"PREVALENCE OF NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS* AMONG SCHOOL CHILDREN OF AN URBAN AREA – A ONE YEAR STUDY"

By

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This is to certify that the dissertation entitled **"Prevalence of nasal carriage of *Staphylococcus aureus* among school children of an urban area – A one year study"** is a bonafide research work done by ***REG NO: BI0115001.***

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LIST OF ABBREVIATIONS

S. aureus	-	*Staphylococcus aureus*
CA-MRSA	-	Community- acquired methicillin resistant *Staphylococcus aureus*
HA-MRSA	-	Hospital-acquired methicillin resistant *Staphylococcus aureus*
GMRSA	-	Gentamicin and methicillin resistant *Staphylococcus aureus*
SBA	-	Sheep blood agar
MSA	-	Mannitol-salt agar
MHA	-	Mueller- Hinton agar
Scc	-	Staphylococcal cassette chromososme
SSTIs	-	Skin and soft tissue infections
CDC	-	Centre for Disease Control
TSST	-	Toxic-shock syndrome toxin
CRF	-	Coagulase-reacting factor
ETA	-	Epidermolytic toxin A
ETB	-	Epidermolytic toxin B
MIC	-	Minimum-inhibitory concentration

ABSTRACT

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the most prevalent and clinically significant pathogen which is associated with both nosocomial and community acquired infection.

As a microbial flora, nasal colonization of *S. aureus* act as an endogenous reservoirs for infections in the colonized individuals as well as a source of cross-colonization for others in the community. *S.aureus* is notable for its rapid acquisition of antibiotic resistance, particularly for Methicillin. Previously restricted to hospitals, *Methicillin Resistant S.aureus(MRSA)* has now emerged in the community, known as *CA-MRSA* which has been recognized as a major global problem. Healthy school going children under the age of 16yrs are identified as the potential carriers of *S.aureus*,including *MRSA*.

OBJECTIVES:

1. To estimate the prevalence of nasal carriage of *S.aureus* among school children (5-16yrs of age)

2. To estimate the prevalence of '*Community-acquired Methicillin resistance (CA-MRSA)'* from the isolates of *S.aureus*

MATERIAL AND METHODS

The study was carried out for the period of one year from January 2016 to December 2016.

A total of 468 children of age group 5-16 years from 15 different schools of urban Belgaum (Belagavi) located under Ramnagar Urban Health Centre(UHC) of KLE University's Jawahar Lal Nehru Medical College (JNMC) were enrolled for the study. Children suffering from skin and soft tissue infections, consuming or have recently consumed antibiotics for any indications, and having history of hospitalization within the last 12 months were excluded from the study.

Nasal swabs from anterior nares with sterile pre-moistened cotton swabs were collected and cultured for isolation and identification of *S. aureus* and MRSA was detected using cefoxitin disc diffusion method.

Antibiotic susceptibility testing was performed by Kirby-bauer disc diffusion method as per CLSI guidelines for all the *S. aureus* isolates.

RESULTS:

Out of 468 children selected, majority of the children i.e; 41.23% belonged to 9-12 years age-group and females were more i.e; 246 (52.6%) as compared to males i.e; 222 (47.4%).

In the present study, the overall prevalence of nasal carriage of *S aureus* was found to be 30.3% and that of CA-MRSA 6.2%.

The higher percentage of resistance was found against Ciprofloxacin and Erythromycin by both S. aureus and MRSA isolates. Amoxyclav, Clindamycin and Cotriamoxazole were found to be more effective.

CONCLUSION:

Children in Belagavi, South India have a high rate of colonization with *S. auerus*. Nasal colonization of CA-MRSA is comparatively higher at alarming rate among healthy children lacking traditional risk factors for MRSA infection. This study has demonstrated the baseline colonization rate and continued surveillance of this population is necessary to assess the ongoing risk CA-MRSA poses to this community. The high rate of antibiotic resistance to frequently used antibiotics like Ciprofloxacin and Erythromycin is a major concern warranting continued surveillance and antimicrobial stewardship programs to promote judicious use of antimicrobials in the hospital and ambulatory settings. Emergence of Linezolid resistance in community is the fact to be drawn attention and measures to be taken to prevent it for keeping this drug as the reserve for the future in today's antibiotic-resistant era

Keywords: Children, Nasal carriage, CA-MRSA

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the most prevalent and clinically significant pathogen which is the leading cause of wide variety of human bacterial infections worldwide; ranging from mild and self-limiting skin and soft tissue infections (SSTI) to life threatening sepsis and fatal necrotising pneumonia which are associated with significant morbidity and mortality.(1)

S. aureus can be considered normal human flora because approximately a third of the population is colonized by the organism with no associated disease. In addition to the anterior nares, the throat, axilla, groin and perirectal area, and non-intact skin are sites colonized by *S. aureus*. About 30% of healthy non-institutionalized individuals are colonized asymptomatically with *S. aureus* in the anterior nares.(2) *S. aureus* carriers have a higher risk of infection than non-carriers and they are an important source of spread of *S. aureus* strains among individuals.(3) It is well known that endogenous *S. aureus* colonization is closely related to nosocomial infection and is associated with healthcare-associated bacteremia, increased risk of wound infection after surgery, and mortality among long-term healthcare facility patients.(4)

Various studies suggest that healthy school going children under the age of 16yrs are potential carriers of *S.aureus*.(5)

S. aureus has a remarkable ability to acquire resistance to antibiotics. Due to rapid adaptation to the selective pressure of antibiotics by various mechanism and indiscriminate use of antibiotics to treat the infections caused by this organism have led to increase in emergence of resistant strains.(6) Infections caused by antibiotic resistant *S. aureus* have occurred in epidemic or pandemic waves over the past 60 years.Within 10 years after penicillin was introduced for use in humans, in many cases it was no longer effective for treatment of *S. aureus* infections due to acquisition of plasmid-encoded beta lactamase. Penicillin-resistant *S. aureus* became pandemic throughout the late 1950s and early 1960s. Among these, *Methicillin-Resistant S. aureus (MRSA)* is reported to cause 40-60% of nosocomial infections, known as *Hospital-Acquired MRSA infections (HA-MRSA)*.

Methicillin-resistant *S. aureus* (MRSA) was first reported in 1961, two years after the antibiotic was introduced to treat penicillin-resistant *S. aureus*.MRSA spread worldwide over the next several decades and is now endemic in most hospitals and healthcare facilities of industrialized countries. Knowing the fact that Methicillin-resistant *S. aureus* (MRSA) isolates are resistant to all available penicillins and other β lactam antimicrobial drugs and thus posing therapeutic challenges. Many of these *MRSA* strains susceptible only to non-betalactams like glycopeptides are becoming resistant to the currently available antibiotics also.(7)

These resistant strains (MRSA) were once confined largely to hospitals, other health care environments, and patients frequenting these facilities. Since the mid-1990s, however, there has been an explosion in the number of MRSA infections reported for populations lacking risk factors for exposure to the health care system.(8) Prevalence of *MRSA* infections has increased among healthy population (particularly children) even without prior healthcare contact. These strains are recognized as new *MRSA* strains often called as ***Community-Acquired MRSA (CA-MRSA)*** strains.

CA-MRSA was traditionally regarded as MRSA strains causing infection in previously healthy young patients without prior healthcare contact, susceptible to most non-β-lactam antimicrobial agents, and carrying Panton-Valentine leucocidin (PVL) genes and staphylococcal cassette chromosome (SCCmec) types IV or V. In order to fulfil the current Centers for Disease Control and Prevention (CDC) definition of CA-MRSA, MRSA must be identified in the outpatient setting or <48 h after hospital admission in an individual with no medical history of MRSA infection or colonisation, admission to a healthcare facility, dialysis, surgery or insertion of indwelling devices in the past year.(3)

CA-MRSA, like all strains of *S. aureus*, is transmitted by direct contact with the organism, usually by skin-to-skin contact with a colonised or infected individual. However, fomites contaminated with CA-MRSA might have a role in transmission in some settings. The Centers for Disease Control and Prevention in Atlanta, USA, have proposed five factors or five Cs of MRSA transmissions such as crowding, frequent skin-to-skin contact, compromised skin integrity, contaminated items and surfaces and lack of cleanliness. Thus, the subgroups of population, especially military recruits, children in day care centres are at high risk of infection.(3)

CA-MRSA strains, often being multi-drug resistant, display enhanced virulence, spreading more rapidly in the community and causing more severe illness than traditional *HA-MRSA*.(9) World over community acquired methicillin resistant *S. aureus* (CA-MRSA) has been recognized as a cause of skin and superficial infections, haemorrhagic pneumonia, sepsis, deep tissue infections and gastroenteritis. In India,CA-MRSA has been reported in pyoderma and other infections like necrotizing pneumonia.(10)

Interestingly, CA-MRSA has entered the hospital as a highly virulent clone. Representing an additional burden, CA-MRSA is responsible for around 30 percent of *S. aureus* infections in hospitals.(10) The reasons for the emergence of CA-MRSA strains as a cause of HAI are unknown. The influx of CA-MRSA carried on patients, visitors and HCWs into hospitals present several challenges. This may be attributed to the ability of CA-MRSA to cause infections in previously healthy individuals in the absence of the selective pressure of antimicrobial agents. So, There is a growing urgency to study the colonization behaviour of CA-MRSA. Screening for nasal colonization of MRSA in all the patients coming to health-care facilities and its eradication from identified carriers before getting admitted is a good idea to prevent the spread of more virulent CA-MRSA strains in hospital setting.

There are various methods available to detect these resistant strains ranging from conventional culture and cefoxitin disc diffusion method to molecular methods like Polymerase chain reaction(PCR), VITEK, & MALDI-TOF. Timely detection permits nasal mupirocin therapy to eradicate nasal carriage and thereby control of MRSA.

Emergence of the virulent and drug-resistant *CA-MRSA* strain in the community is worrisome fact. It is necessary to identify the carriers of this strain and take appropriate action, by controlling them at the source itself, to prevent their spread.

Considering that more representative community based studies are required to assess the true prevalence of CA-MRSA in various parts of the country the present study was aimed at assessing the prevalence of nasal carriage of *S. aureus* (including CA-MRSA) among school children of an urban area.

AIMS AND OBJECTIVES

1. To determine the prevalence of nasal carriage of ***Staphylococcus aureus*** among school children of age 5-16yrs.

2. To detect the ***Community-Acquired Methicillin Resistance(CA-MRSA)*** from the isolates of *S.aureus*

REVIEW OF LITERATURE

Staphylococcus aureus (S. aureus) is a common cause of numerous local and disseminated infections of the various systems like musculocutaneous, respiratory, circulatory & urinary tract system.(11)It is an important pathogen associated with nosocomial and community acquired infection.(1)As a nosocomial pathogen, it is a leading cause of bacteremia and infective endocarditis (IE) as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device related infections.(12)

As a microflora, *S. aureus* colonizes the various parts of body mainly anterior nares in nose, axilla, throat and groin where it acts as an endogenous reservoir for infections in the colonized individuals as well as a source of cross-colonization for others in the community.(1) Approximately 30-40% of human population has been estimated to be colonized with *S. aureus* The throat and nares show higher colonization detection as single sites ranging from 50.5% to 89.7%.(8)

A nasal carrier contaminates his or her hands by nose picking or any contact with nose. Thereby, transmitting the organism in the course of their daily activities. Skin contact with skin is the most significant mode of transmission. So hand washing is of utmost importance in preventing the spread of MRSA infection. Direct contact transmission involved contact of body surfaces and physical transfer of *S. aureus* to the host from an infected or colonized person.(13)

Nasal colonization by *S.aureus* has been considered an important risk factor for infections that could threaten a carriers's life.(1)The predisposing factors for acquisition of invasive diseases by *S. aureus* nasal colonization was reported as overcrowded population (large family size in small house), exposure to health care facilities, poor socio-economic conditions, and prior antibiotic usage.(10)

Morality rate of patients due to infections caused by *S. aureus* was 80% till penicillin was introduced in the 1940s. Again as early as 1942, strains of *staph aureus* producing penicillinase enzyme causing resistant to penicillin were detected in hospitals. Within two decades, 80% of *Staph aureus* isolates were penicillin resistant.(14)

S. aureus is notable for its rapid acquisition of antibiotic resistance, particularly for Methicillin, a semisynthetic penicillinase resistant penicillin introduced in 1960 for treatment of penicillinase producing strains of staph aureus. Previously restricted to hospitals known as *Hospital-acquired Methicillin Resistant S. aureus (HA-MRSA)* strains, *Methicillin Resistant S. aureus (MRSA)* has now emerged in the community, known as *CA-MRSA* which has been recognized as a major global problem.(10)

The past two decades have witnessed two clear shifts in the epidemiology of *S. aureus* infections: first, a growing number of health care associated infections, particularly seen in infective endocarditis and prosthetic device infections, and second, an epidemic of community-associated skin and soft tissue infections driven by strains with certain virulence factors and resistance to β-lactam antibiotics.(12)

HISTORY AND EMERGENCE OF MRSA

S. aureus has always been the most versatile human pathogen posing therapeutic challenges since long. The mortality rate due to infections caused by this organism was about 80% till the introduction of benzyl-penicillin in early 1940's. But the problem was temporaliy solved and as early as by 1942, the continuous use of this agent led to the selection of resistant strains which started producing penicillinase (β-lactamase) enzyme. Within two decades, 80% of both hospital and community-acquired *S. aureus* isolates were penicillin resistant.(5)

Again temporary relief was obtained with the introduction of a semisynthetic penicillins like Methicillin and cloxacillin in 1959 which were penicillinase resistant penicillins used for the treatment of penicillinase producing strains of S. aureus. Even before it was widely used, a few naturally occurring methicillin resistant Staphylococci were reported which however did not cause any major clinical problems until in 1961, methicillin-resistant *S. aureus* (MRSA) suddenly started spreading around hospitals in U.K. (12)

MRSA is a specific strain of the staph aureus bacterium that has developed resistance to all penicillins. It includes resistance to methicillin and other narrow-spectrum β-lactamase resistant penicillin antibiotics.

HA-MRSA

MRSA infections occuring in people who had been in healthcare set-ups is known as healthcare associated/ Hospital acquired MRSA (HA-MRSA). It is associated with invasive procedures like surgeries, insertions of intravenous tubing or implantation of artificial joints. HA-MRSA can be defined as “the MRSA isolate which is associated if the entry criteria of hospitalization for more than 72 hours before culture of the sample taken was met and if in the year before the present admission to hospital, the patient had undergone any of the following: hospitalization, surgery, had been living in a long-term care facility and hemodialysis or peritoneal dialysis or at presently had indwelling catheters or other percutaneous devices”.(8) Methicillin-resistant *S.*

aureus (MRSA) infections account for 40-60% of all nosocomial *S. aureus* infections in many centres across the world.(1)

Prior to the mid-1990s, investigation into the epidemiology of MRSA was limited largely to the health care setting because it was rare that MRSA strains would infect otherwise healthy people.

CA-MRSA

There is another type of MRSA infection occurring in the wider community, among healthy people. This form is called as community associated MRSA (CA-MRSA). It usually begins as a painful skin boil and spreads by skin-to-skin contact. Population who are younger (like children), live in overcrowded conditions (athletes, prisoners, and soldiers), have certain ethnic backgrounds, are intravenous (IV) drug users, and who are men who have sex with men are at high risk for CA-MRSA.(8) Various study suggests that healthy school going children under the age of 16yrs are potential carriers of *S.aureus*,including *MRSA*.(15)

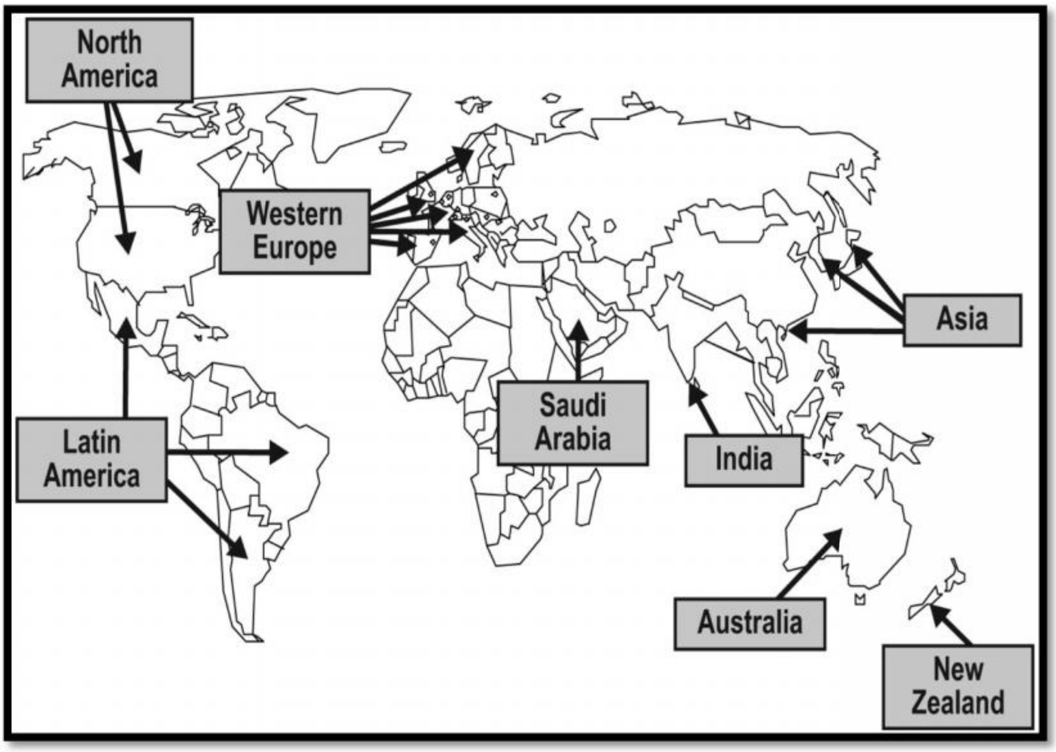
In 2000, Centre for Disease Control (CDC) of USA created a case definition for a CA-MRSA infection. CA-MRSA is considered as “any MRSA infection diagnosed for an outpatient or within 48 hours of hospitalization if the patients lacks the following health-care associated MRSA risk factors: hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient.” (8)

Comparison of HA-MRSA and CA-MRSA

	HA-MRSA	**CA-MRSA**
Area affected	Blood stream, surgical site, implant site	Skin, lungs
Person affected	Immunocompromised, resident in long-term facilities, recent surgery, recent hospitalization, dialysis	Young, otherwise healthy, no recent hospitalization: Anyone
Transmission	Skin to skin contact, contaminated equipment, poor hygiene	Skin to skin contact, cuts or scrapes, crowded area, poor hygiene
Genetic	SCC mec I,II & III	PVL, SCCmec type IV &V
Recombination treatment	Debridement, antibiotic, education	Incision and drainage, antibiotic, educating, hygiene
Prevention	Good hygiene, infection control, staff education, careful antibiotic administration, follow up	Good hygiene, proper wound care, no-touch technique, education, no item sharing
Screening/ diagnosis	Test not required; skin or nasal swab taken; PCR for rapid testing; screen high risk patients; CLSI recommended testing	Testing not recommended; testing wound drainage; testing colonization culture not recommended.

By the end of 1970, these resistant strains appeared in Australia and disseminated to the other parts of the world. Since then, worldwide epidemics of *S.aureus* diseases have been recognized over the years.

Fig 1 Global outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* infection, 1997–2000(16)



Outbreaks of MRSA have been reported in variety of settings including hospitals, long term care facilities, outpatient clinics, as well as in the community. Community MRSA strains have several distinguishing characteristics enabling them to more readily colonize and infect otherwise healthy hosts.(17)

Several epidemiological studies were carried out which found increased morbility and mortality from MRSA strains of *S. aureus* compared with those from MSSA. (18)

Kuehnert reported for 2001-2002 in USA, MRSA prevalence estimated to be 32.4%.(19)

MRSA is endemic in India and is a dangerous pathogen for hospital acquired infections. Reports of MRSA incidence found as 24% in 1996 in Vellore(20) are there. MRSA prevalence increased from 12% in 1992 to 80.83% in 1999. (21) Verma et al (21) also commented it to be of same order in Mumbai, Delhi and Bangalore.

Anupruba et al (22) (2003) reported the incidence of MRSA in eastern Uttar Pradesh to be 54.85%.

An Indian pilot surveillance programme (2013) for detection of MRSA was conducted. This programme led to the detection of MRSA prevalence of 41%.(23,24)

Fig 2 Map showing members of Indian network for surveillance of antibacterial resistance (INSAR) 2013 (24)

Places marked with red dots are INSAR members whose data are included in the study. Places marked * are INSAR members whose data are not included in the study.)(24)

Mechanism of methicillin resistance and its detection

The 2.1 kb mecA gene is located on a mobile genetic element called the Staphylococcal Cassette Chromosome mec (SCCmec). The resistance of staph aureus to methicillin, a semi-synthetic penicillinase resistant penicillin is caused by the presence of mecA gene which encodes the 78 kDa penicillin-binding protein (PBP) 2a. Beta lactam antibiotics cannot bind to the PBP2a and so synthesis of the peptidoglycan layer and the cell-wall of the organism are not able to continue.(5)

Strains of MRSA that possess mecA gene are either heterogenous or homogenous in their expression of resistance. The heterogenous expression occasionally results in false interpretations of borderline minimal inhibitory concentrations as susceptible. So accurate diagnosis is needed. 4 the mecA gene detection tests based on PCR correctly identify even the most heterogenous of strains. Detection of mecA gene or PBP2a is considered the gold standard for MRSA confirmation. It should be considered the gold-standard for methicillin resistance.(25)

Most clinical laboratories rely on disc diffusion testing for the detection of methicillin resistance in staph aureus. Out of various disc diffusion methods, cefoxitin disc diffusion test is recommeneded for detection of MRSA. Cefoxitin, a cephamycin antibiotic is a potent inducer of mecA regulatory system and is shown to be more accurate for detection of mecA gene mediated methicillin resistance in staph aureus. Cefoxitin disc diffusion zones are much easier to read than those of oxacillin. It is due to the hazy oxacillin zones formed which are misinterpreted as susceptible to oxacillin.(26)

PCR is the most well-developed molecular technique up to now. It has a wide range of clinical applications including specific or broad-spectrum pathogen detection, evaluation of emerging novel infections, surveillance, early detection of bio-threat agents and antimicrobial resistance profiling.(27) But PCR based detection of MRSA is expensive and is not readily available in routine laboratories in developing countries like India. (29)

Staphylococcus aureus

Definition

Gram positive, non motile, non spore forming cocci that characteristically divide in more than one plane to form irregular clusters. Facultative anaerobes that grow better under aerobic conditions. Can grow in the presence of 10% sodium chloride and between 10-40^{0}C. Usually form catalase, susceptible to lysostaphin Cell wall contains peptidoglycan and teichoic acid.(28) The diamino acid present in the peptidoglycan is L-lysine. G+C content of DNA-30 To 39 mol %.

Habitat

10-40 % adults not in hospital carry staphylococcus aureus in the anterior nares. Hosspital patients have a higher carriage rate which may increase significantly during prolonged stay in the hospital. (29), Prepubertal children show a higher carrier rate than adults. (29) In addition to the nasal membrane and skin, staphylococcus aureus to a lesser extent are also found in the perineum, gastrointestinal tract and the genital tract. (30)

Morphology

Staphylococcus aureus are non-motile, non-flagellate, asporogenous bacteria that form spherical cells with an average diameter of 0.5 – 1.5 micron. They form grape like clusters. In liquid medium pairs and short chins may occur and be mistaken for streptococci. They stain well with most aniline dyes uniformally and are gram positive in young cultures.

Culture characters

Staphylococcus aureus grow well in unenriched medium like nutrient agar, producing, smooth, circular, opaque, often golden yellow colonies thereby also termed coloquiolly as "**Golden staph**". Colonies are 1-2 mm in diameter after overnight incubation at 37^oC. The colonies are butyrous and easily emulsifiable. Heavily capsulated colonies may be highly **mucoid** and run down the surface of the agar on prolonged incubation. In nutrient broth most strains grow moderate to dense turbidity with a powdery deposit.

On Mackonkey's agar the colonies are small and pale pink after 24 hours and deep pink after 48 hours.

Colonial pigmentation is variable and influenced by growth conditions. Pigment production is enhanced with age and storage in refrigeration. **Increased pigmentation is produced in the dark.** The ability to form pigments may be lost irreversibly on repeated subcultures anaerobically or prolonged culture in broth. (31) Non pigmented variants appear to be less virulent.(32) They produce yellow pigments, which are **triterpenoic carotenoids** located in the cell membranes.

Metabolism

Metabolism is oxidative and fermentative. Glucose is metabolised to pyruvate via the Embden-Meyerhof (EM) glycolytic pathway and /or hexose monophosphate (HMP) pathway.(28) The major end products are acetate and carbon-dioxide. Acetoin is a minor end product.

Growth occurs at 10 and 45^{0}C but usually most rapid between 30 and 37^{0}C. It occurs over a wide range of pH (4-9) and is optimum at pH 7.0-7.5.

An organic nitrogen sources is required i.e. certain amino acids and B group of vitamins.(28) For anaerobic growth uracil and a fermentative carbon source (pyruvate) are also required.

Resistance

Usually killed by a temperature of 60^{0}C in 30 min, but resistant to freezing and drying. Can survive upto 6 months on threads. Tolerant to high concentration of salt (12.5%) and sucrose. Susceptible to chlorhexidine, phenol and hexachorophane but resistant to mercuric chloride. Highly susceptible to aniline dyes (Crystal violet 1:500000) and fatty acids.(33)

Resistant to lysozyme but susceptible to lysostaphin.(28)

Biochemical properties

Major efforts have been devoted to the search for enzymatic tests to separate staphylococci and micrococci.

Acid is produced aerobically and anaerobically from glucose, lactose, maltose and mannitol. Acid is produced aerobically, from fructose, galactose, mannose, ribose, sucrose, trehalose, turanose and glycerol. Acid is not produced from arabinose, xylose, cellobilose, sorbitol, inositol, salicin, adonitol, dulcitol and raffinose.

Catalase positive and oxidase negative. Nitrate are reduced to nitratases (nitrate reductase) and nitritases with the formation of ammonia. (34)

Esculin and starch usually not hydrolysed. Forms a gelatinase, digests casein rapidly in the presence of serum.Produces elastolases.(34)

Urease is produced. Acid and alkaline phosphatases are produced. Arginine is hydrolysed. Ornithine and Lysine is not decarboxylated. A iipase is procuded leading to production of opacity in egg yolk medium. (34)

Acetoin, alpha-hemolysin, fibrinolysin and clumping factor production are common. Typical strains possess 2-NAD-dependent lactate dehydrogenases. (34)

Most strains produce termonucleases. Antibody to this nuclease is specific indicator of staphylococcal diseases in patients with osteomyelitis. (34)

Cell wall composition and antigenic structure

Peptidoglycan and teichoic acid are the major components of the cell wall. The chemical composition and immunological properties of these polymers have been extensively used for taxonomy. Other antigenic components which are generally present in the cell wall include protein A, clumping factor and type specific antigens.

i) Peptidoglycan

The interpeptide bridge in the peptidoglycan of staphylococcus aureus typically contains oligo-glycine peptides. This pentaglycine interpeptide bridge is typically attached by lysostaphin. (35)

An agglutination procedure has been developed to distinguish between the wall of staphylococci and micrococci.

ii) Teichoic Acid

N-acetyl glucosamine substituted by ribitil, teichoic acid, is a typical constitutent of the walls of staphylococcus aureus. This has serological and chemotaxonomic significance.The teichoic acid is antigenic.

iii) Protein A

This antigen was designated protein A in 1964.(36)It is present regularly in the human strains of staphylococcus aureus and is responsible for the agglutination of most strains by all normal human sera. This is a non-specific reaction, since protein A non-specifically combines with the Fc portion of human IgG.(37) This protein was first described by Verwey in 1940.(38) It forms the basis of Co-agglutination reaction.

(iv) Clumping Factor

In 1908, Much observed that staphylococci that coagulated plasma (tube test) were also clumped by it (slide test). It was found almost exclusively in strains that form the ‘true’ or extracellular coagulase. Some heavily capsulated strains can give a negative slide coagulase reaction presumably because the clumping factor is covered by extracellular polysaccharide.(39)

(v) Type specific antigens

Initially Cowan (40) recognized 3 serological types of staphylococcus aureus known as Cowan I, II and III strains. Subsequently about 30 type specific antigens have been recognized.(28) These antigenic markers are stable but require absorption of sera by heterogenous strains whic remove the common agglutinins and reveal a number of type specific antibodies.

Toxins

Staphylococcus aureus produces the following toxic substances –

1. General toxic substances which contribute to the pathogenesis of local or systemic inflammatory diseases. Such as the haemolytic toxins.

2. Specific toxic substances, which contribute to the pathogenesis of local or systemic inflammatory diseases. Such as the enterotoxins and the epidermolytic toxins.

Haemolytic toxins

Four types are produced which damage the red blood cells and thereby produce hemolysis.

i) Alpha lysine

It is a protein consisting of a polypeptide chain with carbohydrate residues. The purified toxin has haemolytic, lethal, dermonecrotic and leucocidal properties. Treatment with formalin inactivates the toxin but its antigenicity persists. 86-95% of staphylococcus aureus produces alpha toxin.(28) Heating at 70% inactivates the toxin, but further heating at 100^0C causes partial reactivation. This toxin also affects smooth muscles, blood vessels, nerve sheaths and kidneys.

ii) Beta lysine

This is a phospholipase C and acts specifically on sphyngomyelin and lysolecithin. It causes hemolysis of sheep red cells only when cooled to 4^0C (hot-cold phenomenon). Magnesium is required for its optimum activity.

iii) Gamma lysin

Initially gamma lysin was thought to be the alpha-2 component of alpha lysine. Now it is been shown to be immunologically distinct. Antibodies to this toxin may be detected in patients with deep seated staphylococcal infection.

iv) Delta lysine

It is a polypeptide of low molecular weight. It lyses red cells from wide range of animals. Also acts on leucocytes and other tissues. Antigenic in nature but the antibodies produced do not appear to neutralise the toxin.

Leucocidin

It acts selectively on leucocytes. First described by Panton and Valentine in 1932 (41)it consists of 2 components designated fast (F) and slow (S). It acts by activating the cell membrane associated phospholipase A 2.

Enterotoxins

Enterotoxins causes food poisoning. There are 7 antigenically distinct types, A to E. The C antigenic type can be subdivided into C1, C2, and C 3. These are proteins with a molecular weight of 26-30 X 10^3. The amino acid sequence of these toxins have been worked out.(42)These toxins are resistant ot the action of proteases such as trypsin, chymotrypsin and pepsin. It is inactivated by heating at 100^0C.

Enterotoxins probably cause emesis by stimulating the vagus nerve. These are pyrogenic, mitogenic, produce thrombocytopenia and hypotension.

Epidermolytic toxin

Staphylococcus aureus strains from exfoliative lesion in humans form toxins that separate layers of the epidermis from underlying tissues. This toxin production is particularly associated with phage group II strains (especially of phage type 71). (43)

There are 2 epidermolytic toxins ETA and ETB. Both are proteins with molecular weight 30000 and 29500 respectively. ETA is heat stable and the production is chromosomally controlled. ETB is heat labile and plasmid mediated.

Toxic shock syndrome toxin (TSST)

TSST is a protein of molecular weight of 22000. It is associated with menstruating women using tampons. It is also called Enterotoxin F (44)and pyrogenic exotoxin C. (45)

Enzymes

Coagulase

It is an extracellular substance produced by staphylococcus aureus which coagulates plasma. It is called free coagulase to differentiate it from ‘Bound coagulase’ or clumping factor. It was first described by Loeb in 1903-04 and confirmed by Much in 1908. Coagulase is probably a large molecule.

Coagulase clots plasma in the presence of fibrinogen and a plasma constituent called coagulase reacting factor (CRF). There is no conclusive proof of the role of coagulase as a virulence factor.

Apart from staphylococcus aureus, coagulase is also produced by staphyloccus intermedius and staphylococcus hyicus.

CRF reacts with coagulase to form staphylothrombin which converts fibrinogen into fibrin. Differences in the reaction of plasma from various species to coagulase is due to the presence and specificity of CRF.

Staphylokinase

It is a protein with a molecular weight of 13000. Three forms of staphylokinase are produced by staphylococcus aureus. Its activity can be demonstrated by the appearance of zones of clearing immediately around colonies on plates containing heat precipitated fibrin. (28) Staphylokinase digests fibrin. It activate plasminogen to form plasmin in human. It has a strong negative correlation with the production of betalysin. (28)It also exhibits the 'Muller' phenomenon.

Hyaluronidase

Nearly all strains of staphylococcus aureus produce hyaluronidase. However its role in pathogenicity is uncertain.

Production of antibiotic substances

Both coagulase positive and coagulase negative strains of staphylococci produce bacteriocins which have wide range of activities. (41)

The bacterocin widely studies is formed by those staphylococcus aureus strains which produce epidermolytic toxins. Production is controlled by a gene on the plasmid which also controls the production of epidermolytic toxin B. (28) Bacteriocins are not used for typing of staphylococci.

Clinical significance

Staphylococcus aureus broadly causes two forms of diseases –

1) Acute inflammation:

This usually begins at or near the point of entry. It generally remains localised but occasionally extends and leads to generalized infection.

2) Acute toxaemia:

It results from the absorption of extracellular products formed by the organism at a local lesion, at a carrier site or even outside the body.

In general it can be said that the staphylococcus aureus are parasites of the body surface and their ability to cause disease appears to be only an incidental factor. Though they have the capability to multiply in almost all tissues, they are denied the opportunity by the host defence mechanism. Notwithstanding that staphylococcal diseases are very common because the conditions that permit the organism to gain access to the tissues are so many and so frequent. Although sporadic skin lesions in human are common, which is of self infectious nature, in certain artificial conditions sepsis may become a communicable disease. The most common and well studied example is hospital acquired staphylococcal infections, which arise as a result of creation of populations of susceptible patients in which surgical and medical treatment provide opportunity for the infection of many persons by a single strain. (28)

Disease of the skin and subcutaneous tissue

a. Pustular diseases

These are the commonest diseases caused by staphylococcus aureus and the characteristics lesion is a boil. The ability to cause boils appear to be strain specific character which may at times be associated with a particular phage typing pattern. For example the 52, 52 A, 80 and 81 complex of the phage group I strain which were prevalent in hospitals in the 1950s and 1960s showed exceptional capability to cause boils not only in patients but also in the hospital staff. (46)Hospital associated phage gp III strains are rarely lysed by phage 71, unlike other strains. Staphylococcus aureus from boils are nearly always egg yolk positive.

The type of staphylococcus found in the lesion generally corresponds with the patients nasal carriage especially in styes and sycosis-barbae.(47) But the infecting strains is absent from nose in almost 50% of cases with boils. Intrafamilial spread of infection is common and in almost 50% of the cases, the offending strains can be isolated from another member of the family. (48)

Between 1950 and 1960 sepsis was common among family contacts of persons recently discharged from hospitals. After 1960, with the decreasing prevalence of 52, 52 A, 80 and 81 complex in hospitals, it is no longer a common phenomenon.

Host factors predisposing to boils by staphylococcus are numerous namely, pressure and minor traumas, diabetes and other mechanical factors. Babies, adolescents and patients with higher nasal carriage of the organism are more susceptible to development of boils. (49)

b. Exfoliative diseases

Exfoliative staphylococcal lesions have been variously named. All these diseases are characterised by stripping of the superficial layers of the skin from the underlying tissues by the aciotn of the epidermolytic toxins. Parker et al in 1958 suggested that most of the strains responsible belong to phage group II and were lysed only by phage 71. They never gave rise to opacity in egg yolk or split Tween 80, but many of them formed a characteristic bacetiocin. In the later reports Azavedo and Arbuthnott showed that about 25% of the strains did not belong to phase gp II. (50)

i) Scaled skin syndrome (Lyell 1956, 1967) and earlier known as Ritter's disease (1878). The onset is abrupt, extensive areas of skin are exfoliated. Large flaccid bullae appear. In 1-2 days skin become wrinkled and peels off on light stroking (Nikolsky's sign). It mainly affects babies and young children. The epidermolytic toxin produced can act at a distance from the site of multiplication of staphylococcus.(51)

ii) Scarlantifini form rash in association with staphylococcal scarlet fever has been described. The cause is the epidermolytic toxin produced at a distant site.(52)

iii) Bullous impetigo (Engman 190) and pemphigus of the newborn (Almquist 1891). It contains blebs which contain fluid. Generally associated with poor hygienic conditions. Pemphigus is a neonatal emergency. These blebs result from the action of epidermolytic toxin.

iv) Necrotic fasciitis, is associated with general toxaemia and there is wide-spread destruction of subcutaneous tissues. Exfoliative lesions can also be seen with toxic shock syndromes. (28) Similar lesions are also seen when staphylococci infect skin lesion of non-microbial origin like chicken pox, and scabies. (28)

c. Sepsis in newborn infants

Minor skin infections are very common in new born babies and tend to occur in epidemics in neonatal nurseries. In the absence of epidemics, sepsis rate is 10-25% in hospitals and 10% in home deliveries. (53)

d. Other superficial skin infections

i) Cervical adenitis

ii) Acute mastitis of nursing mothers

iii) Sepsis in wounds and burns, staphylococci are the commonest cause of sepsis in accidental as well as clean surgical wounds.

iv) Infection of urinary tract. Mostly hospital associated. Many follows catheterization and operations on bladder or prostate. These infections may lead to serious complications like bacteremia or spread to kidney and perinephric tissues.

e. Staphylococcal pneumonia

Staphylococcus aureus invades the lung form the bloodstream giving rise to multiple abscesses. The following types are seen

i) Staphylococcal pneumonia in infants. It affects the sick and premature babies with high mortality and long term complications like empyema, lung abscess or pneumatocoele. In 1950s, epidemics were caused by the 52, 52 A, 80 and 81 complex.

ii) Post influenza pneumonia is the main cause of death in healthy people after influenza A infection. [2] Generally it is a superimposed infection afflicting the already damaged mucosa of the lower respiratory tract by the virus.

iii) Pneumonia in debilitated adult hospital patients. Most such causes are hospital acquired. The isolation of staphylococcus aureus is a poor guide to frequency of staphylococcal pneumonia in such cases. In a study carried out in Britain 6% of cases have been found to be suffering from staphylococcal pneumonia during autopsy conducted on patients dying in hospital.

f. Osteomyelitis

Staphylococcus aureus is the commonest cause of osteomyelitis and is usually accompanied by bacteremia.

g. Generalized infections

Staphylococcus aureus may spread from a superficial lesion, through tissue planes, lymphocytes or blood stream. However, transient bacteremia are common in the absence of any local source which may predispose to osteomyelitis and renal abscesses.

Endocarditis develop in many cases where there are no obvious primary source of infection and therefore difficult to eradicate.

The major complications of staphylococcal septicaemia are thrombocytopenia, glomerulonephritis, DIC and at times peripheral gangrene. (28)

i) Tropical pyomyositis

Large abscesses develop in voluntary muscles with scattered myonecrosis. The cause and the mode of spread of staphylococci is uncertain. 60% of the strains are found to be belonging to phage group II with typing pattern 3A/3B/3C/55/71.

ii) Staphylococcal toxic shock syndrome

In 1978, a syndrome characterised by high fever, headache, confusion, vomiting, diarrhoea, subcutaneous oedema, hypotension and shock was described by Todd et al. (54)Staphylococcus aureus was isolated from local septic sites but blood culture was negative.

Between 1979 and 1980 similar illness was reported from USA affecting young women. There was a strong association between menstruation and use of intravaginal tampons.

Davis et al reported the incidence at 6/10000 menstruating women per year. (29)

Shilevert et al in 1979 (45) and Burgdoll in 1981 (42) described a toxin which is now universally called ‘Toxic Shock Syndrome Toxin’ (TSST).

iii) Diarrhoea and vomiting

These are caused by ingestion of food in which staphylococcal aureus has multiplied and formed enterotoxins, diarrhoea and vomiting can also be caused by use of antibiotics which destroy the normal gastrointestinal flora and be replaced by *Staphylococcus aureus* strains resistant to multiple antibiotics. (55)

Typing method

1. Bacteriophage typing

The present phage typing system was described by Wilson and Atkinson in 1945, (56) and later modified by Williams and Rippon in 1952. (57) The staphylococci are characterised by different lysis patterns obtained with a set of phages.

The basic set of typing phage consists of 23 different Staphylococcus aureus bacteriophages. Virulent phage cause lysis of staphylococcus that they are able to infect and produce a clearing in the lawn of growth in a nutrient agar plate.

It is possible to subdivide typing phage into lytic groups and allocate the staphylococci into corresponding phage groups. (58)

Strains of Staphylococcus aureus can be allocated to three main phage groups I, II and III. Group I contains hospital epidemic strains eg type 80 and 52A/79. Group II contains strains causing minor sepsis outside hospitals and strains of type 71 and 3B/3C/55, most commonly causing blistering lesions and producing epidemolytic toxins. Group III includes animal strains, many of the heteroresistant hospital strains like type 47/53/75/77, and enterotoxin producing strains. Group IV phages are restricted for typing isolates from animals. An new group V has been added for strains restant to the typing phages. A nmber of new typing phages (94, 96) have been typing thus added to the typing set to increase typability. (Parker 1953)

2. Serotyping

Two serological methods have been described (Oeding and Billet). (59) The use is limited by the shortage of sera and expertise in their use.

3. Plasmid profile and restriction endonuclease analysis of plasmids (Archer 1984). (60)

4. Ribotyping

5. Antibiogram

6. Gel electropheoresis Fingerprinting: Using [^{35} S] methionine labelled markers

Pathogenicity

Experiments conducted on humans and animals (Goshi) (61) indicate that the following local conditions favour the appearance of a staphylococcal lesion on the skin.

i) Vasoconstriction

ii) Necrosis

iii) Acute inflammation due to another microorganism

iv) A hypersensitive reaction (Gurmohan Singh) (62)

v) Presence of foreign bodies

vi) Cell-associated and extra cellular virulence factors

Phagocytosis

Various abnormalities of the phagocytic process are of primary influencing in causation of staphylococcal sepsis .Jobs syndrome I and chronic granulomatous deseasae are the result of such defects.

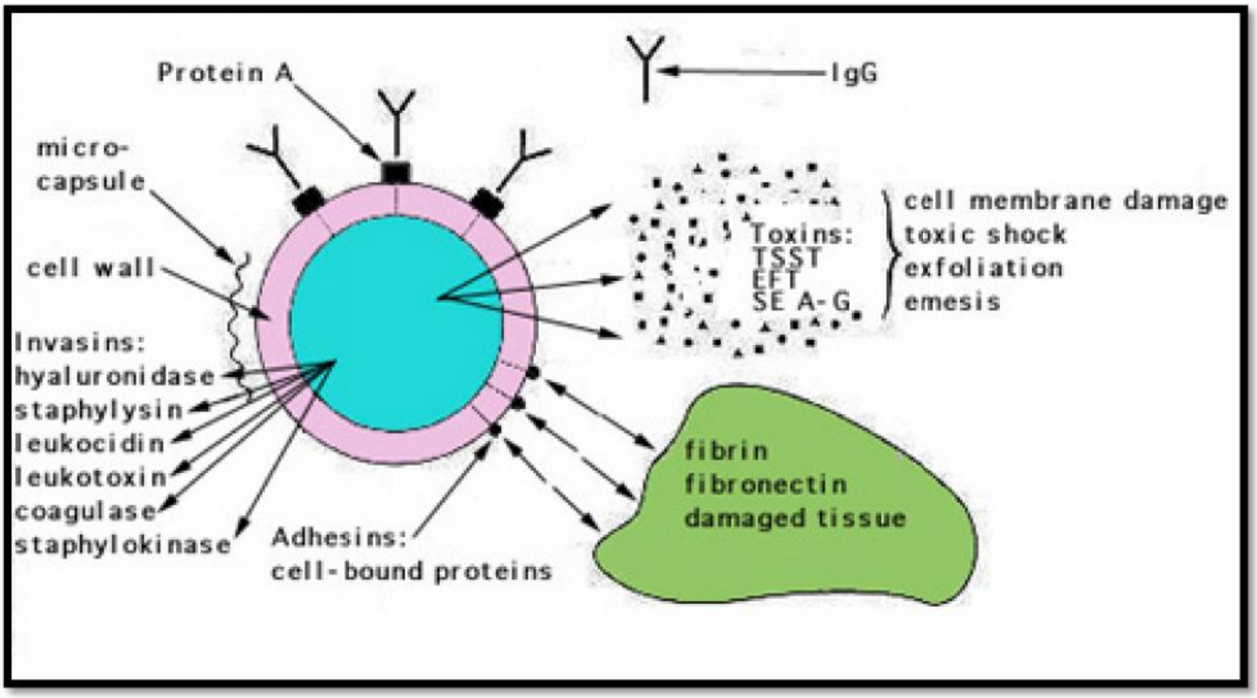
Cellular virulence factor

i) The addition of a dead organism or foreign body to a living innoculum favours production of lesion. This is attributable to aggressive cell wall factor.

ii) Capsular polysaccharide have an antiphagocytic action

iii) Continuous passage of Staphylococcus aureus in rabbits greatly enhances its virulence .

Figure- 3 Various virulence factors of *S. aureus*



Role of extracellular toxins

a) Alpha lysine contributes to necrosis and delayed mobilization of leucocytes. Lethal effects also contributes.

b) Extracellular factors have chemotactic activity of leucocyte (Russel) 67 especially on polymorphonuclear cells.

Sources and routes of infections

1. Endogenous sources – majority of the infections arise from endogenous sources which not only include sporadic skin lesions but also serious disorder like post influenza pneumonias, neonatal sepsis and surgical wounds.

2. Exogenous source - Staphylococcus aureus from other person may reach a susceptible site in a patient and cause a lesion. This occurs mainly in hospitals. The organism is carried by direct contact or through air and contaminated objects.

In view of the above, carriage of staphylococcus aureus assumes importance in causing self infection as well as infection in another person.

Carriage

1. 30-50% of population carry the organism in the anterior nares at any moment (53)

2. Familial predisposition to nasal carriage, a possible genetic influence (Noble et al) (48)

3. Age

a. Newborn – 60-70% at home and 80-100% in hospital

b. 1-4 years – 20%

c. Above 5 years – 30-50%

d. Elderly – 20-25%

4. Persistent carriers – 20-35% of all nasal carriers, usually harbour the same strain for months or years.

5. Intermittent carriers – 30-70% short terms persistent carriage of a single strain which is lost. It may be acquired due to contact a persistant carrier

6. Never carriers – 10-40%

7. Colonization by one strain generally prevents implantation of other strains.

8. Non susceptible individuals beomce highly susceptible if given an antibiotic such as oxacillin

9. Throat carriage – 4-64%

10. Facial carriage – 10-20%

11. Skin carriage – 10%

12. Hand - 40%

13. Perineum – independent of nasal carriage, rate 10-20%

All these are rich sources of airborne dissemination

Airborne dissemination

Most nasal carriers are profuse dispensers of staphylococci into the air (White). (63) Heavy perineal carriage almost invariably dispense large number of organisms. The dispersion from skin surfaces increases during physical exertion. Thom and White in 1962 observed that there was little dispersal by patients during surgical incision of minor staphylococcal lesions, but heavy contamination of air occurred when soiled dressing were distributed. (64) Treatment of carrier with an antibiotic to which his/her strain of *Staphylococcus aureus* is resistant may increase the profuseness of carriage and the heaviness of dispersion of the organism. Staphylococci rapidly accumulate on clothes and beddings where they survive for long periods and can tolerate dust and initial drying (Lidwell & Lowbury) (65) which are disseminated as airborne particles during bed making. These particles with a median diameter of 12 nm remains suspended in air and carried for considerable distance by convectional air current. They also pass easily through surgical gowns and ordinary clothing.

Dissemination by contact

Direct contamination of the clothing of hospital staff by contact with patients is of significance.(27,66) In major hospital outbreaks the index case almost always are patients or house-staff physicians, thereby dubbing such spread of infection as “house staff-patient transfer circuit” (67)

Antimicrobial susceptibility

Staphylococcus aureus are inherently sensitive to many antimicrobial agents. However, most strains are now resistant to penicillin. At present strains causing hospital associated infections are highly resistant to methicillin and oxacillin both stable to staphylococcal penicillinase. These methicillin resistant Staphylococcus aureus (MRSA) are able to colonize patients and hospital staff and cause major epidemics in high risk unit like neonatal nurseries, burn units, ICUs and other. (68)

Emergence of antibiotic resistance (69)

Before the antibiotic era of medicine began some 50 years ago, the prognosis for patients with severe Staphylococcal infections used to be extremely poor. The introduction of penicillin into clinical use in early 1940s, however, brought about a dramatic reversal in this situation. (70) Very serious Staphylococcal infections like septicaemias arising out of accidental and operative wounds, burns and other serious skin infections, could be treated effectively. But this phase of effective penicillin therapy was disconcertingly shortlived.

By the year 1946, it was estimated that about 60% of hospital isolates of Staphylococcus aureus in UK had developed penicillin resistance (38,71,72) which necessitated the introduction of other antimicrobials, namely tetracyclines, chloramphenicol and macrolides. These too were similarly attended by the emergence of resistant organisms. Ominously enough, the strains which had acquired resistance to these newer antibiotics were also usually resistant to penicillin through the production of a beta-lacatmase (Penicillinase).

The result thus, was the creation of organisms with a wide spectrum of resistance and a marked ability to survive and be spread in the hospital environment. (73)

With the introduction of semisynthetic B lactamase resistant penicillin, such as methicillin and oxacillin, there was a general decline in the prevalence of multiple resistant Staphylococcus aureus in the early 1960s. Although about 1% of the isolates still exhibited an inherent resistance to methicillin, (74) they did not pose a serious threat to the overall effectiveness of the antibiotic. By the late 1960s and 1970s, however, strains resistant to semisynthetic β lactams were being isolated with increased frequency in a number of countries. Most of the MRSA strains produced B-lactamase and showed marked resistance to streptomycin, sulphonamides, and tetracyclines. Many of these strains also showed additional resistance to chloramphenicol, erythromycin and fusidic acid or to the aminoglycosides neomycin.

The aminoglycoside, gentamicin was introduced in the year 1964. For a long period of 10 years, this drug remained resistance free. In the year 1975 and 1976, a series of outbreaks of hospital infection caused by Staphylococcus aureus was reported which were concomitantly resistant to gentamicin and two other related aminoglycoside kanamycin and tobramycin. Once again, extensive topical use of the antibiotic was implicated in the appearance and spread of these resistant strains. (75)

Subsequently reports of *Staphylococcus aureus* resistant to both gentamicin and methicillin (GMRSA) along with a wide range of other antibiotics including penicillin, tetracycline and streptomycin, came initially from Melbourne, (76) Australia in 1976 and then from other countries like England, Republic of Ireland and USA.(77,78)

During the late 1970 and early 1980s, strains of *Staphylococcus aureus* resistant to multiple antibiotics including methicillin and gentamicin were increasing responsible for outbreak of hospital infections in countries around the world. In many instances, these outbreaks were associated with individual wards or units, with neonatal, ICU, and burn units being particularly susceptible. In many of the hospitals, the organism involved, often possessed the capacity to counter almost all the antimicrobial agents available to the clinician. The persistence and propensity for spread of these organisms posed a very serious clinical problem involving many patients in many hospital. The first such major example of MRSA hospital outbreak is of a tertiary care hospital at Dublin, USA in 1964.

The newer fluorinated-4-quinolones like ciprofloxacin, pefloxacin and ofloxacin were found to be effective during 1984 to 1989. (79) During the 90s resistance to ciprofloxacin and pefloxacin (PRSA) was reported worldwide. A majority of PRSA are also resistant to methicillin and gentamicin. (80) A hospital study (Blumberg et al 1991) has demonstrated an alarming rate of development of ciprofloxacin resistance from none to 79% over a 1 year period. (81)

Rifampin, primarily an antituberulous drug is now being increasing used against heteroresistant staphylococcus aureus either singly or in combinations with other drugs like vancomycin or cefamandole. Occasional isolates have been reported to be resistant to rifampin.

Clindamycin, a macrolide, in a recent reappraisal in UK have been shown to be useful second line agent against Staphylococcus aureus infections. Rate of resistance against MRSA strains was found to vary between 20-30%.

Vancomycin, an older antibiotic discovered more than 30 years ago is presently the drug of choice for infections caused by MRSA strains. Resistance to a vancomycin has only rarely been reported. This drug has been introduced in India very recently.

Other antistaphylococcal agents under evaluation

1. Fosfomycin

An early stage cell wall biosynthesis inhibitor is active against MRSA. A synergistic effect of cefamenadole with fosfomycin against MRSA have been reported. less than 10% of MRSA strains are presently resistant to fosfomycin.

2. Aminocylitols

Arbekacin is highly effective against MRSA. Two percent arbekacin resistance has been reported from a hospital in Japan.

3. Streptogramin

Composed of two compounds which interact synergistically. RP-57669 and RP-54476 derived from pristinamycin IA and pristinamycin IB. The synergistic action of the 2 compounds appear useful in treatment of septicaemia. Gilbert el al have advised against the use of this drug if resistant to lincomycin.

4. Paldimycin

A mixture of two antibiotics chemically unrelated to any other group.

5. Daptomycin

A peptocide, it has been found to be effective as vancomycin in an animal model of MRSA causing pneumonia.

6. Newer macrolides

Clarithromycin, azithromycin, roxithromycin and josamycin are already in clinical use and exhinit a resistance of 10-30%.

7. Linezolid

Use of local antibiotics in management of carriers state

Topical antimicrobials like bacitracin, tetracyclines, chlorhexidine, azelaic acid, nitrofurazone, silver sulfadiazine, and mupirocin have been tried. Of late mupirocin calcium ointment have been shown to be effective and generally safe. Such topical applications have sometimes failed, due to side effects, emergence of resistance and recolonization.

Virulence of MRSA

Various studies conducted during the 80s suggested that multiple drug resistant Staphylococcus aureus strains are probably less virulent to the MSSA strains. However, other studies countered this, reporting that the intrinsic virulence of MRSA was at least equal to the MSSA strains.

Genetics of resistance

The development of powerful techniques in molecular biology and advances made in bacterial genetics have shed much light on the underlying genetic factors which have resulted in the emergence of these antibiotics resistant strains.

The appearance of antibiotic resistant Staphylococcus over the last 40 years has been regarded as an inevitable genetic response to the selective pressure imposed by antimicrobial therapy. The often rapid emergence following the introduction of an antibiotic into clinical use illustrates the ability of microbial population to readily adapt to changes in their environment.

When antibiotic resistance was first encountered among bacteria, including *Staphylococcus aureus*, it was believed to arise solely by mutation and selection.

Spontaneous bacteterial mutants resistant to certain antibiotics can be generated at a frequency of 10^6 to 10^8 per cell in the laboratory, and it was assumed that analogous events have occurred in natural population to produce resistant organisms. Truly, resistance inherent to *Staphylococcus aureus* to several useful antibiotics including streptomycin, rifampin, fusidic acid and novobiocin, is thought to be derived by chromosomal mutations.

However it was realised that chromosomal point mutation which lead to antibiotic resistance can be harmful to the organism itself and it can and does lead to the creation of less virulent organisms. Secondly, the acquisition of new characters, without affecting the fitness of the bacteria to survive in their natural environment, would be expected to occur over a substantial time span. Therefore, the accumulation of chromosomal mutation as a sole explanation for the rapid emergence of multiresistant bacteria, in an evolutionary sense appeared to be unsatisfactory.

The discovery of the gene transfer and the demonstration that bacteria can acquire additional genetic material in the form of extrachromosomal or plasmid deoxyribonucleic acid (DNA) confirmed the minor role played by spontaneous mutation in the sudden appearance of antibiotic resistant strains. The existence of plasmid DNA molecule was suggested by the transfer of direct genetic unit of resistance between bacterial strains and the irreversible loss of such units from cells at a relatively high frequencies. In the last 2 decades, much evidence has accumulated that in many instances, resistance to antimicrobial agents is due to the presence of plasmids that carry the genetic determinants of resistance.

These plasmids contribute to the success of antibiotics in a variety of ways. Since the genes present on the plasmids are not essential for the survival of the organism, the carriage of plasmid reduce the genetic and physiological load on the organism. These plasmid borne genes can undergo radical evolutionary changes without affecting the viability of the cell unlike the chromosomal genes. They can be transferred to the recipient cells along with the genetic information.

The plasmids contribute towards the development of chromosomal resistance in two ways. Firstly it can integrate with the chromosome in part or in toto. Secondly, plasmids along with bacteriophages can act as vectors for transposons or transposable DNA elements.

In addition, some strains of MRSA have decreased sensitivity to commonly used antiseptics including acridines and quarternary ammonium compounds. These determinants are carried on the gentamicin resistance plasmid. This has a important bearing as regards to formulating rational and effective policy on the use of antiseptic agents in hospitals.

Transfer of resistance genes in staphylococci

Several mechanism exists for the transfer of genetic materials between staphylococci.

1. Transduction

For transduction to occur transducing phage particles need to be produced from teh donor strains through infection with a lytic bacteriophage by the induction of a prophage. Calcium ions are required for the attachment of the phage particles to the cell surface. Therefore transduction as well as lytic phage activity can be inhibited by the presence of chelating agents such as citrate ions.

The main Staphylococcus transducing phages belong to serological group G phage, which include typing phages 29, 80, 53 and 88 toghether with 11, however transduction of gentamicin resistance has been associated with a group F phage.

For many years transduction was believed to be the only mechanism through which Staphylococci could exchange genetic information in vivo.

2. Transformation

The transformation of staphylococcus aureus with a naked plasmid or chromosomal DNA can be achieved in the presence of high concentration of calcium ions with recipient cells that have attained competence. Competence is dependent upon the absence of extracellular deoxyribonuclease, the presence of which precludes the transfer of DNA by transformation under natural conditions and the existence of competence conferring factors provided by superinfecting bacteriophage of induced prophage.

3. Mixed culture transfer and Phage mediated conjugation

It has been observed that resistance determinants encoded on plasmids can be transferred in mixed cultures at very high frequencies (10 resistant transcipients per final donor cell) following overnight incubation and that chromosomal resistance genes can also be transferred, although at a some-what reduced frequency. [128] This has led to the proposal of an alternative mechanism in mixed cultures. This has been referred to as phage mediated conjugation because there is a requirement of a prophage in either the donor or the recipient, together with high cell density and calcium ions.

Lacey postulates that the bacteriophage could be instrumental in altering teh adhesiveness of the cell surfaces, thereby enhancing the clumping of cells and enabling the plasmid DNA to pass directly form donor to recipient. In this process the donor cell need not be lysogenic, unlike transduction.

4. Plasmid mediated conjugational transfer

This mechanism is independent of phage activity and require a viable cell to cell contact. Such self transmissible (Tra+) Plasmids are also able to mobilise or transfer smaller antibiotic plasmids which are independently transferrable by a simple conjugation like process.

The importance of both phage mediated and plasmid mediated conjugation provide attractive mechanisms for the transfer of antibiotic resistance determinants among Staphylococci under natural conditions. The transfer of resistance determinants in mixed culture or the human skin has been demonstrated, thereby suggesting the importance of the body surface, the normal staphylococcal habitat, in the emergence of antibiotic resistant organism.

Plasmid mediated conjugation has been reported to be stimulated in the presence of dry absorben surface; therefore, transfer of resistance might also be expected to take place on surgical dressings, clothing and bedding in hospital environment.

Resistance to methicillin and other beta-lactamase resistance penicillins

The mode of action of methicillin, cloxacillin and cephalothin is similar to that of Penicillin G. There are 4-5 different penicillin binding protein which are enzymes essential for cell wall synthesis. These PBPs; covalently bind with penicillin and are thereby inhibited. Of the various PBPs it is likely that PBP2 and PBP3 are the lethal targets for penicillins.

Resistance to methicillin and other beta-lactamase resistant penicillins could therefore result either from an instrinsic change in one or more PBPs, which provide immunity from all beta-lactam antibiotics or from the synthesis of a new PBP with reduced affinity to beta-lactam antibiotics.

Epidemiology

Risk groups

Patients in tertiary care hospitals, the elderly, those with burns, neonatal nurseries, patients in ICU, surgical wards, immunocompromised state and longer stay in hospital. Previous treatment with several antibiotics also predispose to MRSA infections.

Organisms:

The most commonly implicated organisms belong to phage group III especially types 77, 83A, 84 and 85. However other non-typable strains can also cause outbreaks (eg 88). Phage group V (96) is also known to cause hospital epidemics.

Control measures

The following control measures are advocated.

General measures:

a. To prevent the access of Staphylococcus aureus to susceptible sites from which they can invade the tissues.

b. To lessen the chances that the organism that do reach such sites will cause sepsis

c. To reduce as far as possible the number of human sources of Staphylococcus aureus in the immediate neighbourhood of the patients

Specific measures

1. **Surveillance**

During outbreaks expert surveillance can anticipate the progression of the outbreak and thereby intervene to alter the course of events. Specific surveillance methods include;

a. Inspection of microbiologic results, the monthly survey of accumulated reports, and the identification of infected patients, colonized patients and colonized staff members

b. Colonized patients – monitoring of close contacts like nurses, doctors, other personnel and even visitors.

c. Regular ward rounds to ensure that appropriate isolation and hygienic precautions are being taken

d. Special surveillance for patients who are at high risk

2. **Isolation**

a. Various studies 159, 161 have attested to the crucial importance of isolation in containing the nosocomial spread of MRSA strains in a hospital. Routine isolation is not useful-strict isolation in a single room is necessary. 79

b. Isolation of carriers and discharging them at the earliest.

c. Curtailment of admission of carriers and restricting transfer of such case to another hospital.

d. Isolation of all patients till proved that they are non-carriers.

e. Grouping of patients and staff members during outbreaks.

3. Management of carrier state

a. Use of chlorhexidine solution for hand washing, bathing and shampooing. Other antiseptics like hexachlorophene and povidone iodine has also been proved to be useful.

b. Disinfection of ward and furnitures – phenolics are recommended.

c. Eradication of nasal colonization.

    a. Use of topical antimicrobials – calcium mupirocin ointment has been found to be satisfactory in eradicating nasal colonization

    b. Use of systemic antibiotics – Rifampicin in combination with TMP-SMX or fusidic acid have been tried with variable results, drugs like ciprofloxacin has been tried.

    c. Bacterial interference – instillation of a large innocula of a non-virulent Staphylococcus aureus strain prevents subsequent colonization by other strains of Staphylococcus aureus.

Identification of MRSA strains

During investigation of outbreak of infection, it is essential to identify rapidly and accurately the colonized patient as well as the hospital staff. The failure of which will prolong an outbreak with accompanying cost implications for the laboratory and hospital.

Use of semi-selective isolation media

1. Methicillin blood agar and methicillin nutrient agar

The use of these two media have been recommended by "The combined working party of the Hospital Infection Society and The British Society for Antimicrobial Chemotherapy in their guidelines for the control of epidermic methicillin resistant staphylococcus aureus (EMRSA).

2. Janet et al have reported improved isolation of MRSA strains using methicillin milk agar. On this media MRSA colonies vary in colour from cream to orange. Other media used are Chapman's media with added methicillin.

3. Mannitol-salt agar(MSA) with oxacillin- used for screening of MRSA carriage.

4. Confirmatory test for identification

Kirby-bauer disc diffusion susceptibility testing(82)

a) The methods are described using methicillin disk (5 mcg)/ Cefoxitin disc. Incubating Mueller Hinton agar plates at 30^0C without any added salt for 24 to 48 hours.

b) Incubating MHA plates at 35^0C with 5% sodium chloride added for 18-24 hours.

Hindler suggests that there is a relation between temperature and the quality of MHA. Incubation at 30^0C may provide better conditions for the detection of methicillin resistance when using low quality media, whereas incubation at 35^0C appears to be more desirable when using high quality media.

Spread plate method

Use of oxacillin (6 mcg/ml) on MHA containing 4% sodium chloride and incubated for 24 hours at 35^0C. Pascle Richard et al have reported 100% sensitivity and specificity of the test using 2 mcg per ml of oxacillin, addition of 5% sodium chloride and incubating the plates at 37^0C for 24 to 48 hours.

Agar and broth dilution technique of MIC determination

NCCLS recommends addition of 2% of Sodium chloride incubation at 35^0C for 24 hours with either Methicillin or oxacillin. Oxacillin is preferred because of its stability and reproducibility of its test results.

Molecular techniques

These techniques are aimed at detecting the mec gene present in the MRSA strains which encode for a low affinity penicillin binding protein (PBP 2a). The mec gene and its product PBP2a are never found in MSSA strains.

a) DNA hybridization using a radiolabelled probe

b) Polymerase chain reaction (59,60)

Both systems detect the mec gene with high accuracy and all mec positive strains are methicillin resistant. Few mec negative and positive isolates exhibit a borderline resistance (MIC of methicillin 4-16 mcg/ml). Richard et al have reported that these borderline methicillin resistant strains can be identified by the spread plates technique with 2 mcg/ml of oxacillin incorporated in MHA.

METHODOLOGY

Study Design

A community-based cross sectional study.

Study Setting

The study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College, Karnataka Lingayat Education University(KLEU), Belagavi

Study Population

The study was conducted among children of age group 5-16 years from 15 different schools of urban Belgaum (Belagavi) located under Ramnagar Urban Health Centre(UHC) of KLE University's Jawahar Lal Nehru Medical College (JNMC) . The results of the study is expected to be representative of this age-group of children.

Study Duration

The study was carried out for the period of one year from January 2016 to December 2016.

Eligibility Criteria

Inclusion Criteria

- Children of age-group 5-16 years studying in the schools of urban Belagavi located under Ramnagar UHC of JNMC,KLEU.

Exclusion Criteria

- Children suffering from skin and soft tissue infections such as impetigo, furuncle, cellulitis or other serious medical illnesses.
- Children who are consuming or have recently consumed antibiotics for any indications.
- Children who have history of hospitalization within the last 12 months

Sample Size

Sample size (N) =4pq/ d^2 , where

p= the anticipated prevalence of primary outcome (nasal carriage of *S. aureus* among children aged 5-16yrs),

q=(100-p) and

d= absolute/relative precision (usually 10-20% of prevalence)

For the current study, the sample size calculation follows –

p= 29.3% (Prevalence according to the study done in Udupi)[4]

q= 70.7% (100-29.3)

d (relative precision)= 15% of p = 4.39

therefore,

N = (4 x 29.3 x 70.7) / 4.39 x 4.39= **429.99~ 430**

15% non-response was anticipated in this study due to factors such as non-attendance, refusal & exclusion criteria which is 64.5

- ✓ **Final Sample Size** = 430 + 64.5 = 494.5 ≈ **495**

- Total no. of schools under study in Ramanagar UHC = 15
- Total no. of children in all the 15 schools = 7025
- Sample size = 495
- So, Total no. of children which will be selected for study, Per 100 students = 495/ 7025 x 100 = 7.04 ~ 7
- And Sampling interval for those 7 students Per 100 student = 100 / 7 = 14

Sampling Technique

1) **Population proportion to size method-** to select total no. of students from each school. For example-

   Total no. of children in one school is = 450

   As already calculated, we have to take 7/ 100 students

   So, for this school total no. of students selected will be

   = 450x7/100 = 31.5 ~ 32

2) **Systematic sampling-** to select the students from individual school. After preparing a common list of all the 450 students, 1ststudent was selected from 1ststd among 1st ten students randomly by lottery method.

And since sampling interval is 14, every 14th student onward was selected. Study Instrument

- Since participants are minors, consent from their parent/ guardian and assent from the children were taken.

- A questionnaire was developed along with consent form for collecting the data on following variables–

    - Age & Sex
    - Socio-demographic factors(type of house/ family size)
    - H/O current antibiotic intake (within 1 week)
    - H/O hospitalisation within last 12 months

Ethical Issues

Ethical clearance for the study was obtained from the Institute Ethics Committee

Statistical Analysis

Chi-square test has been used to find the significance of study parameters on categorical scale between two or more groups.

Significant figures

+ Suggestive significance (P value: $0.05<P<0.10$)

* Moderately significant (P value: $0.01<P \leq 0.05$)

** Strongly significant (P value: $P \leq 0.01$)

Statistical software: The Statistical software namely R 3.4.2 & R-studio were used for the analysis of the data and Microsoft Excel 2010 was used to generate graphs, tables etc**.**

Data were entered in Microsoft Excel and analysed by percentage.

Enrollment of partcipants:

The investigator visited 15 schools in urban areas of Belagavi after obtaining necessary permission from authorities. The investigator obtained written informed consent from parents of the enrolled participants and assent from the participants. This was then followed by collection of information on selected socio-economic and health-related variables as well as nasal swab specimen. The samples from nasal swab were then processed for isolation, identification, and antibiotic sensitivity of Staph aureus.

Table 1 below provides the details on the school visited for the study and number of students in the eligible age-groups on the registers of these schools.

Table 1– List of schools visited and number of eligible students in each school

S No	Name of the School	Total students
1	Sharman English Medium School	450
2	Sharman Kannada Medium Primary School	505
3	Madani English Medium School	989
4	Mahila Vidyalaya English Medium School	1399
5	Mahila Vidyalaya Marathi Medium High School	544
6	KLE G A School	357
7	Mahila Vidyalaya Marathi Medium Primary School	295
8	KLE B A Hanchana Primary School	284
9	Govt PHQ Kannada Primary School	268
10	Govt PHQ Urdu Primary School	132
11	Govt Kannada Primary School	100
12	Govt. Urdu Primary School	234
13	Sharman Kannada High School	274
14	NPET English Medium High School	499
15	Maratha Mandal English Medium High School	695
	TOTAL	**7025**

The investigator needed to approach a total of 564 students to enroll the study participants. Of these, 42 students were not present on the date of visits to the school. Of the remaining 518 students, 28 students were excluded due to non-eligibility criteria such as history of hospitalization within last 12 months or present of acute respiratory tract infection on the date of visit, or history of recent exposure to healthcare facilities due to any reason, or history of intake of any antibiotics currently or within last 7 days. Thus a total of 494 students were eligible for selection in the study. But 26 students refused to cooperate and had to be excluded. Finally 468 students were included in the study and were available to provide information on selected socio-demographic variables as well as for collection of nasal swab specimen. All these 468 participants allowed collection of nasal swab specimen.

Table 2 indicates the details of stages of enrolling study participants in the study with reason(s) for non-availability of information or nasal swab specimen.

Table 2 – Details of stages of enrolling students in the study

S No	Parameter	Result
1	Total Students Approached	564
2	Absent during visit for data collection	42
3	Excluded due to non-eligibility*	28
4	Present & Eligible for selection	494
5	Refused to participate	26
6	Included in the study	468
7	Nasal swab specimen available	468
8	Non-response rate	17%

H/O any skin & soft tissue infection, H/O Hospitalization within last 12 months, H/O Recent exposure to healthcare facilities due to any reason, H/O Intake of any antibiotics currently or within last 7 days

Table 3 provides the details of students selected from each of the schools visited. Schools with proportionately larger student list had proportionately higher representation in the study.

Table 3: Details of schools visited and number of students selected

S No	Name of the School	Total students	Students selected
1	Sharman English Medium School	450	32
2	Sharman Kannada Medium Primary School	505	32
3	Madani English Medium School	989	35
4	Mahila Vidyalaya English Medium School	1399	105
5	Mahila Vidyalaya Marathi Medium School	544	35
6	KLE G A School	357	64
7	Mahila Vidyalaya Marathi Primary School	295	21
8	KLE B S Hanchial Primary School	284	20
9	PHQ Kannada Primary School	268	21
10	PHQ Urdu Primary School	132	9
11	Govt Kannada Primary School	100	8
12	Govt. Urdu Primary School	234	18
13	Sharman Kannada High School	274	19
14	NPET English Medium High School	499	34
15	Maratha Mandal English Medium High School	695	15
	TOTAL	**7025**	**468**

Sample collection

Out of 564 school children approached, 468 children were enrolled for the study. After selection of the children and consent obtained from their parents, paired nasal swab were collected each from anterior nares of both the nostrils using sterile cotton wool swabs moistened with sterile normal saline. The swab was rotated in the anterior nares five times and immediately put into tube containing Amie's transport media and sample was transported to laboratory within 2-4 hours.

Sample processing

Swab was inoculated immediately on 5% sheep blood agar and Mannitol-salt agar for culture and incubated at 37^0c for 18-24 hours. Then, Gram's stain was also performed directly from the swab after making smear.

Culture - After incubation for 18-24 hours, characteristic growth of *S. aureus* was identified based on colony morphology ,pigmentation & hemolysis on blood agar and characteristic yellow coloured colonies on Mannitol salt agar.

- **Blood agar**- small (1-2 mm in diameter),white, circular, convex, smooth, moist, golden yellow pigmented or non-pigmented, β-hemolytic or non-hemolytic colonies
- **Mannitol-salt agar –** small yellow coloured colonies

Gram's stain- Gram stain was performed, by Hucker's modification method. Interpretation:- Gram-positive cocci ,about 1µm in diameter, uniformly stained & arranged in grape-like clusters were further processed for identification.

Controls:- Positive – *Staphylococcus aureus* ATCC 25923

Negative- *Escherichia coli* ATCC 25922

Catalase test-

The test was performed by tube method using colony & 3% hydrogen peroxide.

Interpretation- sample giving rapid and sustained effervescence (nascent oxygen) was reported as positive for this test.

Controls:- Positive – *Staphylococcus aureus* ATCC 25923

Negative- *Streptococcus pyogenes*

Coagulase test:

This test was done to differentiate *S. aureus* from other *Staphylococcus* species by both slide and tube coagulase method.

Slide coagulase test: This test was done to detect bound coagulase enzyme present in the cell wall of *Staphylococcus aureus.*

Interpretation:-

Positive - if coarse clumping were visible to naked eye within 10sec.

Negative – No coarse clumping / clumping seen after 10 sec.

Quality control-

Positive - *Staphylococcus aureus* ATCC 25923

Negative – *Staphylococcus epidermidis*

Tube coagulase test - This test was done to detect free coagulase enzyme produced by *Staphylococcus aureus.*

Interpretation:-

Positive – when any degree of visible clot/ stiff gel formation/ clot floating in the medium were seen.

Negative – No visible clots/ only flocculent or ropy precipitate were seen.

Quality control-

Positive - *Staphylococcus aureus* ATCC 25923

Negative – *Staphylococcus epidermidis*

Biochemical test:

Urea hydrolysis test- Christensen's urease agar slope was inoculated with peptone water culture of the isolate and incubated at 37^0c and examined after overnight incubation.

Interpretation:-

Positive – when colour of the media changed from yellow to purple pink.

Negative – No change in colour.

Quality control-

Positive – *Proteus* species

Negative – *Escherichia coli*

Antibiotic Susceptibility testing:

Antibiotic sensitivity was tested by Kirby-Bauer's disc diffusion method using Mueller-Hinton agar(MHA) plate was used. One-two colonies from the culture plate were inoculated into 2ml of peptone water and incubated at 37^0c for 2 hours. Turbidity was compared and adjusted to that of 0.5 Mcfarland's standard ($1.5x10^8$ CFU/ml).

A sterile cotton swab was immersed and rotated in this inoculum while pressing it along the sides of the tube so to remove excess inoculum. The swab was then used to inoculate the plate of MHA by Lawn-culture method, in three different plane to ensure an even and complete distribution of the inoculum over the entire plate. The antibiotic discs were applied within 15minutes of inoculation and plates were incubated for 18-24 hours at 37^0c.

Commercially available Himedia antibiotic discs were used. The potency of discs used and their zone size interpretative standards were according to CLSI guidelines.(82)

The drugs used for sensitivity testing were:-

SL. NO.	Antibiotics	Concentration per disc
1.	Penicillin	10unit
2.	Amoxyclav	20/10 µg
3.	Erythromycin	15 µg
4.	Clindamycin	2 µg
5.	Cotrimoxazole	25µg
6.	Ciprofloxacin	5 µg
7.	Linezolid	30 µg

MRSA detection:(82)

Cefoxitin (30µg) Disc Diffusion Test to detect methicillin resistance-

- A 0.5 McFarland standard suspension of isolate was made.

- Lawn culture was done on MHA plate using this susension and cefoxitin disc were placed on the inoculated surface.

- Plates were incubated at 33-35^{0}c for 24 hours.

- Zone diameter was measured.

- As per CLSI 2017 guidelines-

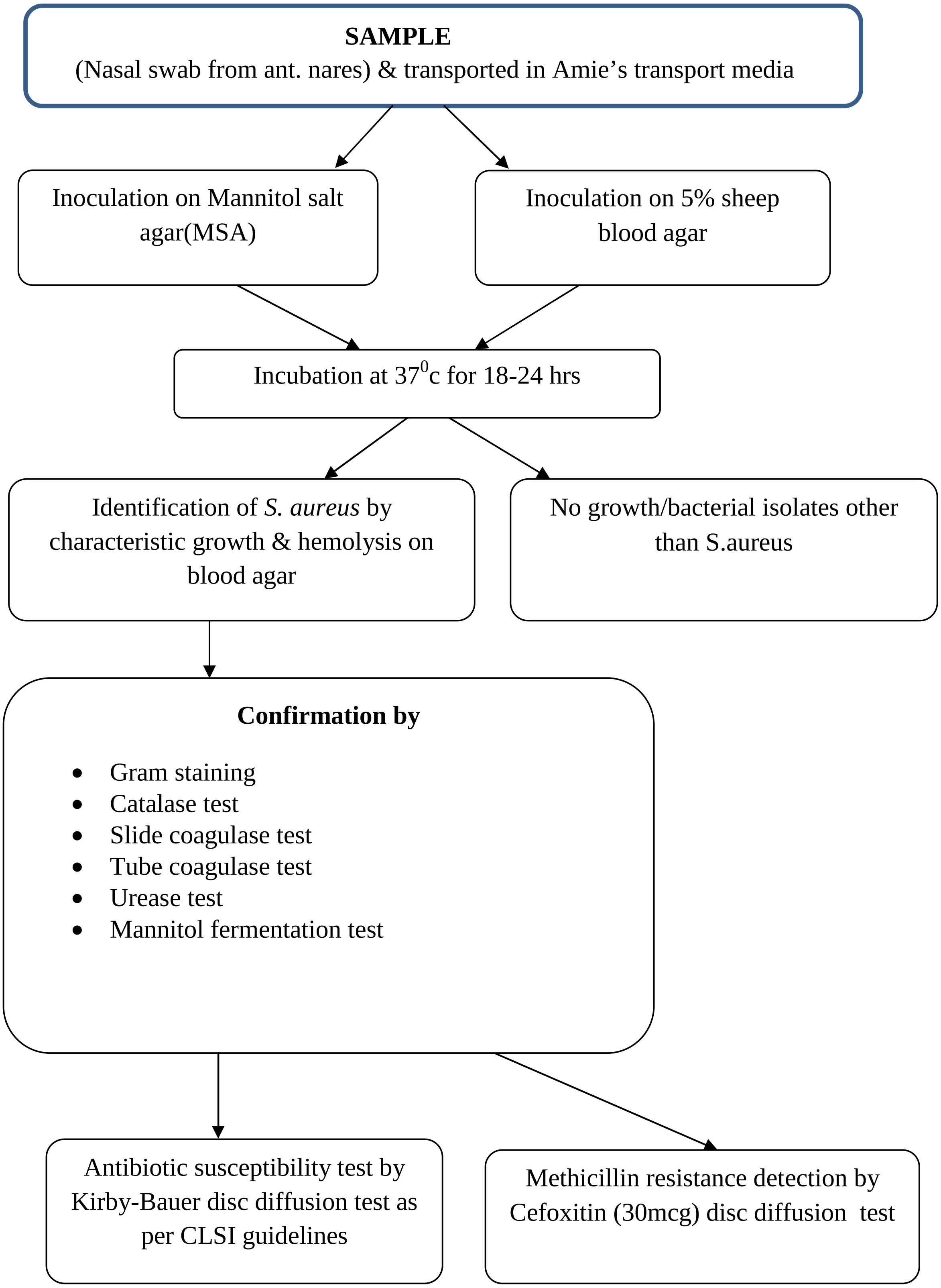
Inhibition zone diameter of ≤21mm= Resistant

Inhibition zone diameter of ≥21mm= Sensitive

-**Positive control** – Methicillin-resistant *S. aureus* (MRSA) ATCC 43300

-**Negative control**- Methicillin-sensitive *S. aureus* (MSSA) ATCC 25923.

Flow chart showing the methodology of sample collection and processing:

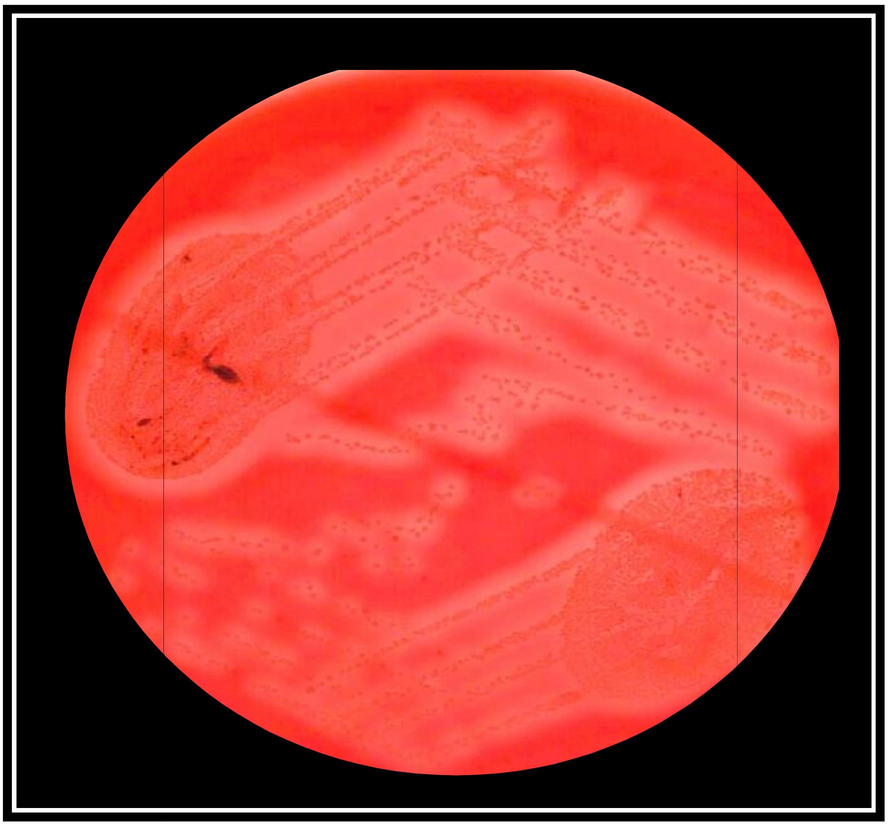


PHOTOGRAPHS

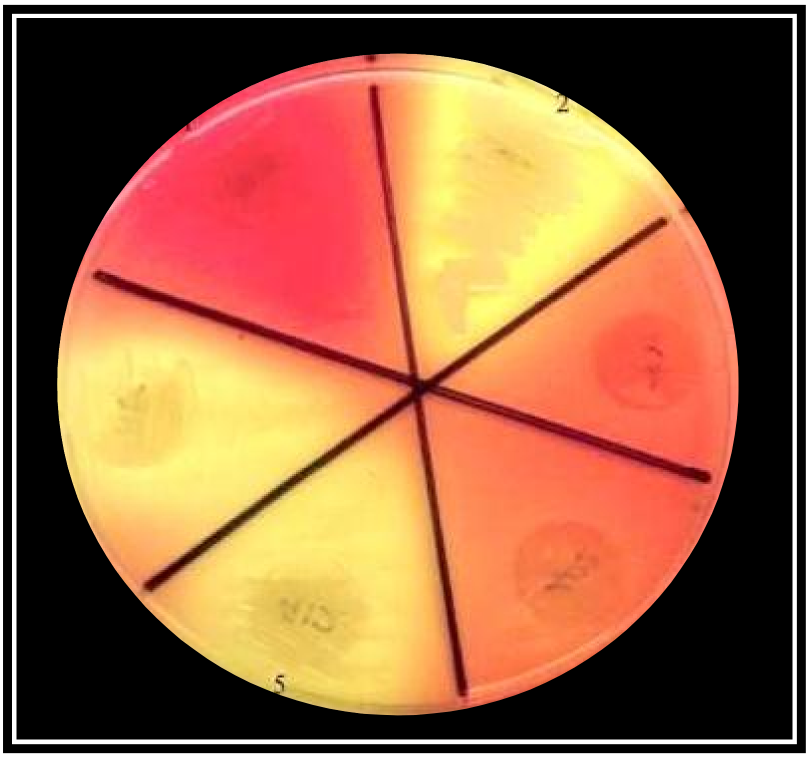
1: Collection of nasal swab from anterior nares of both nostril

2 : Transportation of nasal swab in Amie's transport media

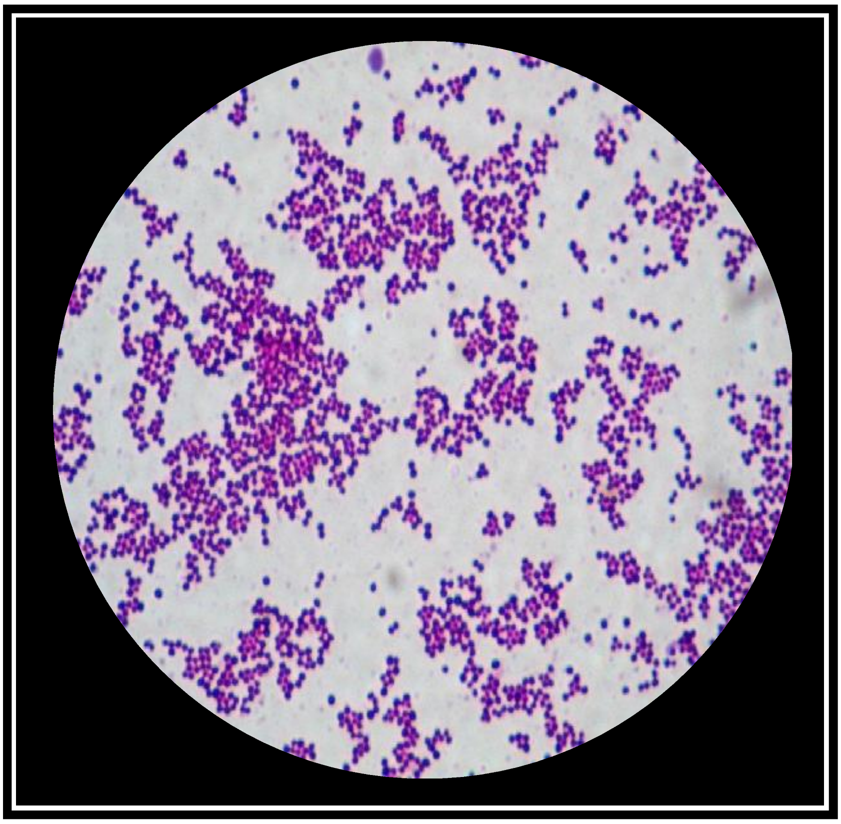
3: Small, circular, white, opaque colonies of Staphylococcus aureus on 5% sheep blood agar



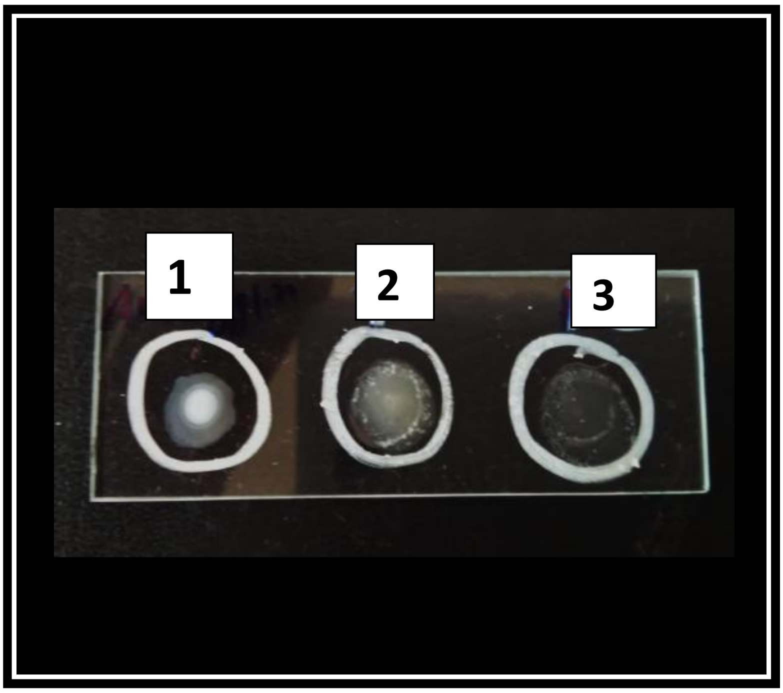
4 : β-haemolysis by colonies of Staphylococcus aureus on 5% sheep blood agar



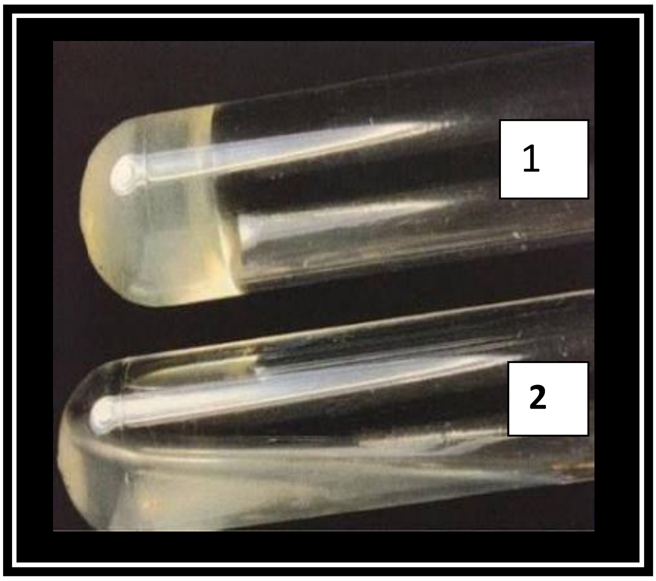
5 : Yellow colonies of Staphylococcus aureus on Mannitol salt agar (MSA)



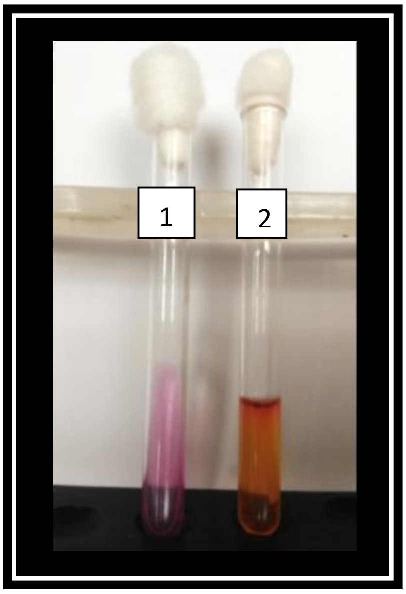
6. Gram-stained smear showing Gram-positive cocci of size approx. 1µm arranged in grape-like clusters



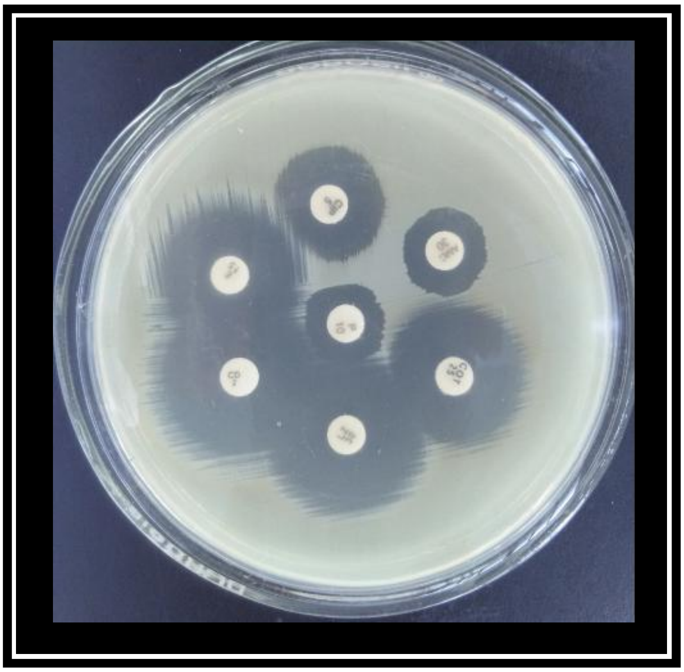
7. Slide coagulase test- 1- control for autoagglutination; 2- test strain positive for test showing visible clumps. 3- positive control



8. Tube coagulase test- 1- test strain positive for test showing clot formation; 2- negative

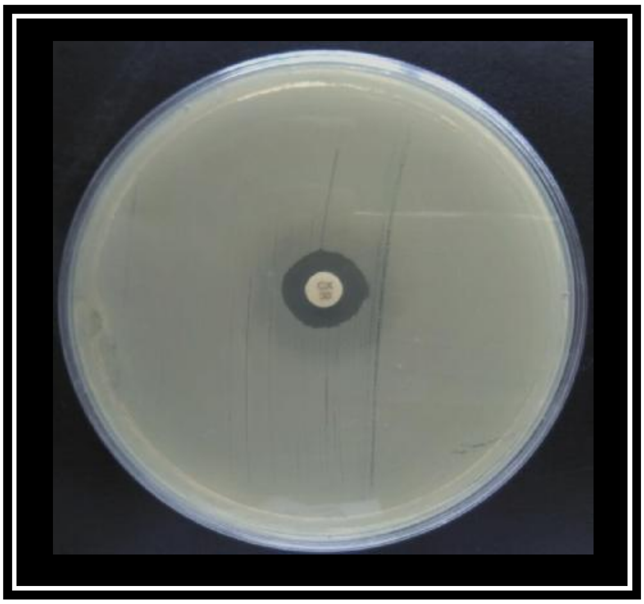


9. Biochemical reactions - 1-Urea hydrolysis test; 2- Mannitol fermentation test

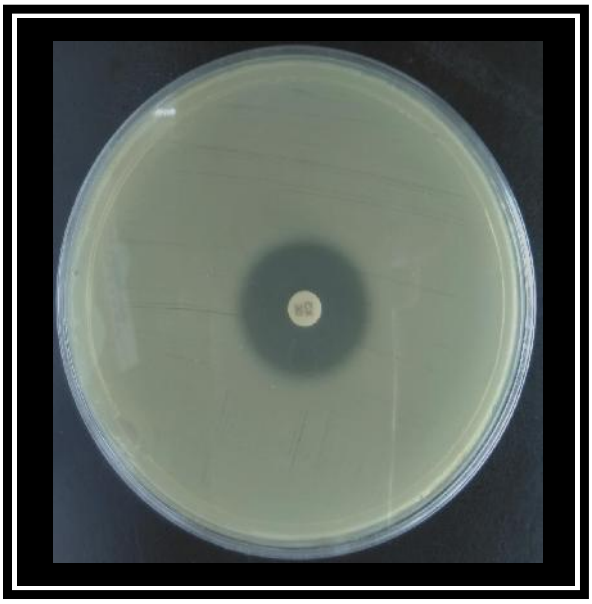


10. Antimicrobial susceptibility pattern of Staphylococcus aureus on Mueller-Hinton agar plate

MRSA detection by cefoxitin disc diffusion



11. Zone size <21mm = Methicillin resistant S. aureus (MRSA)



12. Zone size >21mm = Methicillin sensitive S. aurues (MSSA)

RESULTS

A total of 468 children of age group 5-16 years studying in the schools of urban belagavi were enrolled for the study. Sample was collected and processed for isolation, identification and antibiotic sensitivity of *S. aureus* and finally analysed statistically.

Various socio-demographic details like age, sex, type of house and no. of family members living together were studied and analysed for the association as the risk factors for nasal carriage of S. aureus in the community setting.

Age distribution-

Of the 468 students selected, majority of the students were aged 12, 13 and 14 years (highest number of students aged 13 years, ie 14.32% of total).

We have also presented data of students across three categories of ages, i.e. age-groups of 5-8 years, 9-12 years, and 13-17 years. Here the highest number of participants (41.23%) belonged to 9-12 years age-group. The mean age of the participants was 11.2 years with a standard deviation of 2.8 years.

Sex distribution

Out of 468 students selected in the present study, Female students were a little more i.e; 246 (52.6%) as compared to male students i.e; 222 (47.4%).

Family size

The mean family size of the students was 4.95 with a standard deviation of 0.87. This means that on an average the students belonged to houses with approximately 5 members per family.

Type of house

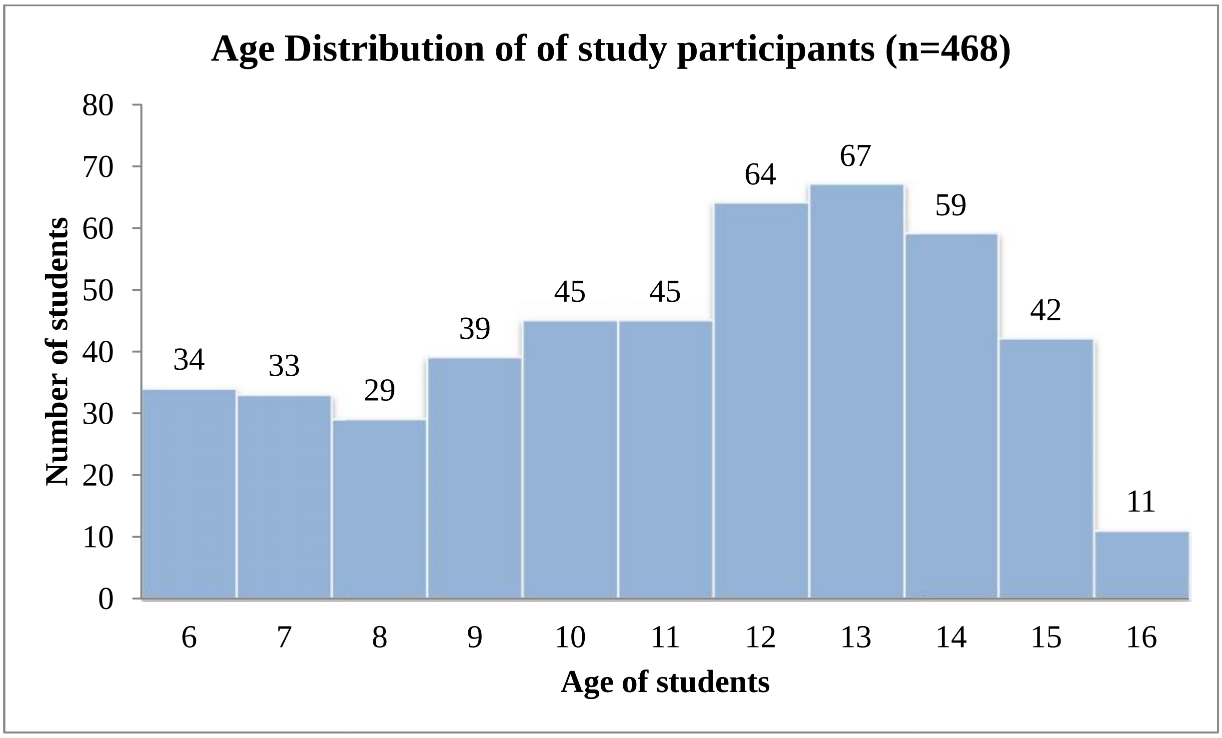
Majority of parents reported in the questionnaire provided to be staying in brick houses i.e; 463(98.9%). Only 5(1.1%) students were reported to be staying in brick house.

Table 4 – Socio-demographic details of Study Participants

S No	Parameter	Result
1.1	Age categories	
	6 years	34 (7.26%)
	7 years	33 (7.05%)
	8 years	29 (6.2%)
	9 years	39 (8.33%)
	10 years	45 (9.62%)
	11 years	45 (9.62%)
	12 years	64 (13.68%)
	13 years	67 (14.32%)
	14 years	59 (12.61%)
	15 years	41 (8.97%)
	16 years	11 (2.35%)
1.2	Age groups	
	5-8 years	96 (20.51%)
	9-12 years	193 (41.24%)
	13-16 years	179 (38.25%)
2	Mean age (SD)	11.2 (2.8)
3	Sex	
	Male (%)	222 (47.4)
	Female (%)	246 (52.6)
4	Mean Family Size (SD)	4.95 (0.87)
5	Type of House	
	Brick (%)	463 (98.9)
	Mud-thatch (%)	5 (1.1)

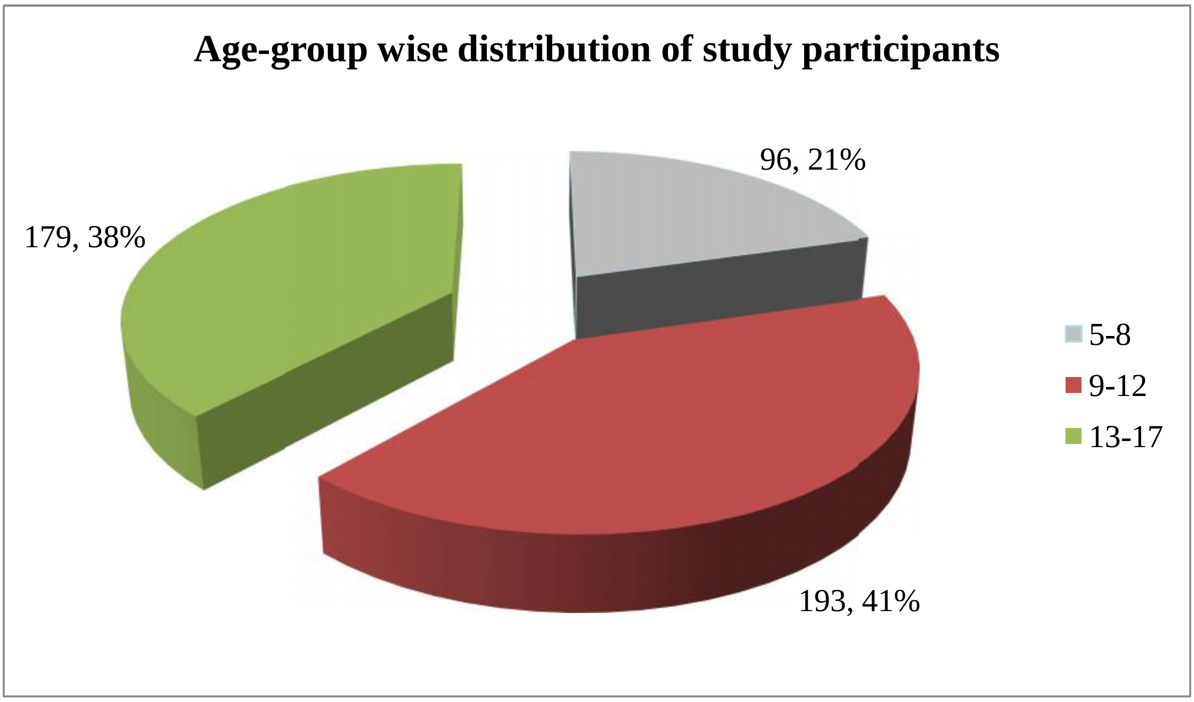
Graph 1 depicts the age-distribution of all the 468 study participants across the various ages.

Graph 1- Histogram showing distribution of age of study participants (n=468)



Graph 2 depicts the age-distribution of all the 468 study participants across the three age-categories of 5-8 years, 9-12 years and 13-17 years.

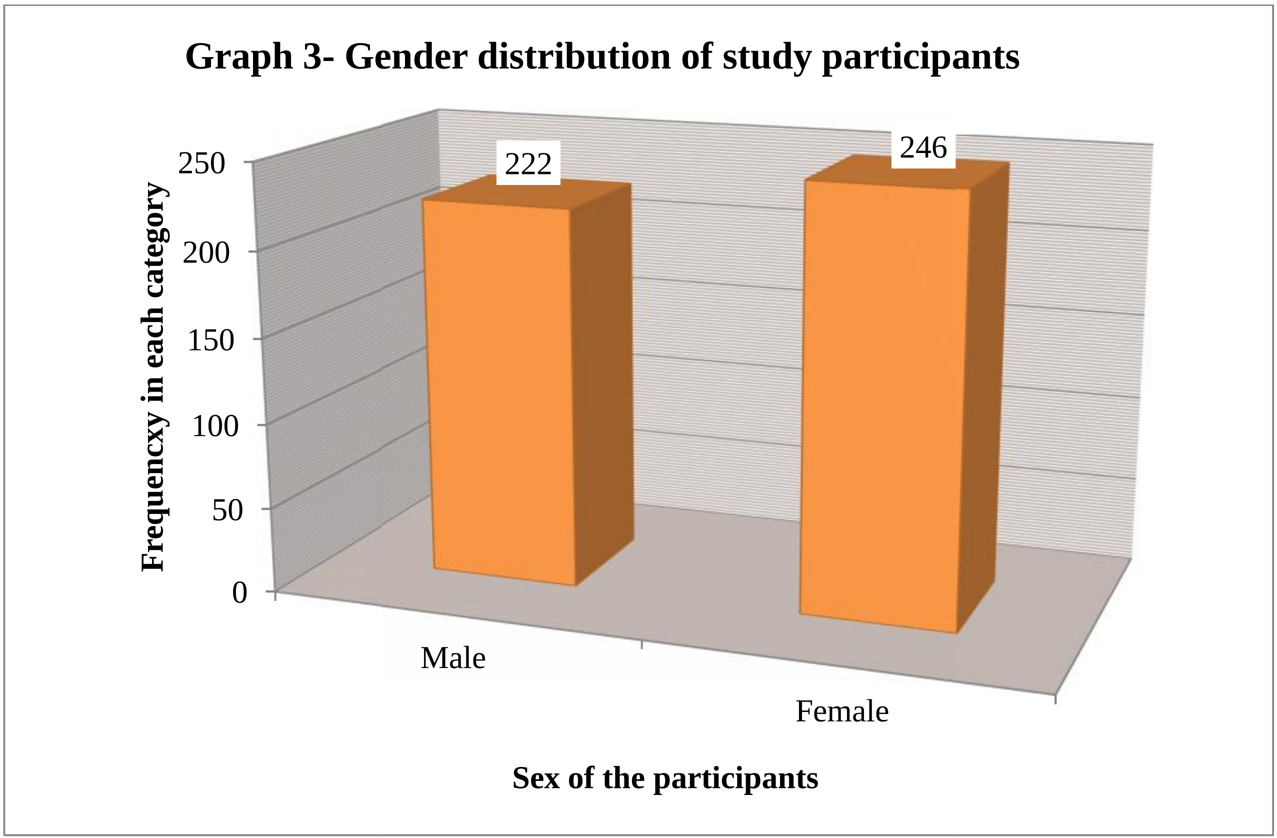
Graph 2- Age-group wise distribution of study participants (n=468)



In the present study,

- The participants belonged to age group 5-16 years.
- Maximum number of students (41%) belonged to the age group of 9-12 years followed by 38% to 13-16 years age group.

Graph 3- Gender distribution of all the participants.



Objective 1- Prevalence of nasal carriage of S. aureus among school children

The primary objective of the study was to find the prevalence of nasal carriage of *Staphylococcus aureus* among the school students. Out of the total nasal specimen of the 468 study participants, *Staph aureus* was isolated from 142. Thus in our study sample, the overall prevalence of nasal carriage of *S aureus* was found to be 30.3% **(Table 5)**. The prevalence among male students was found to be 26.1% and that among female 34.1%.

Table 5 – Prevalence of Nasal Carriage of S aureus (n=468)

S No	Parameter	Male	Female	Total
1	Total samples tested	222 (47.4%)	246 (52.6%)	**468** (100%)
2	Samples positive for *S aureus* (Prevalence of nasal carriage of *S aureus*)	58 (26.1%)	84 (34.1%)	**142 (30.3%)**

Graph 4 shows the prevalence of nasal carriage of *S. aureus*

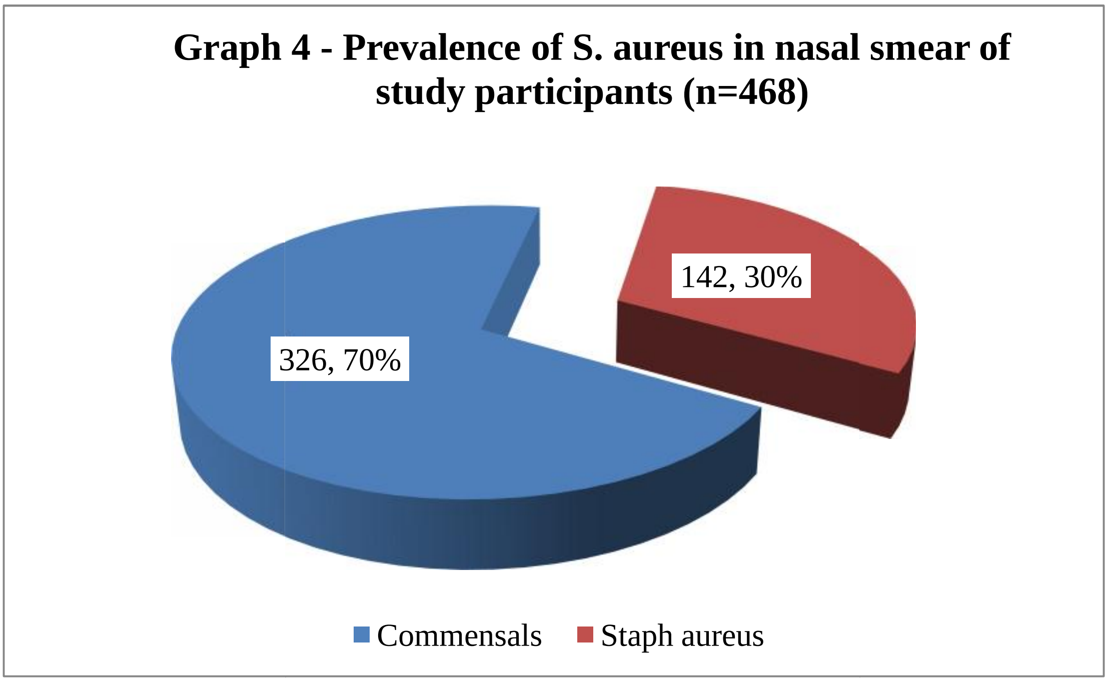
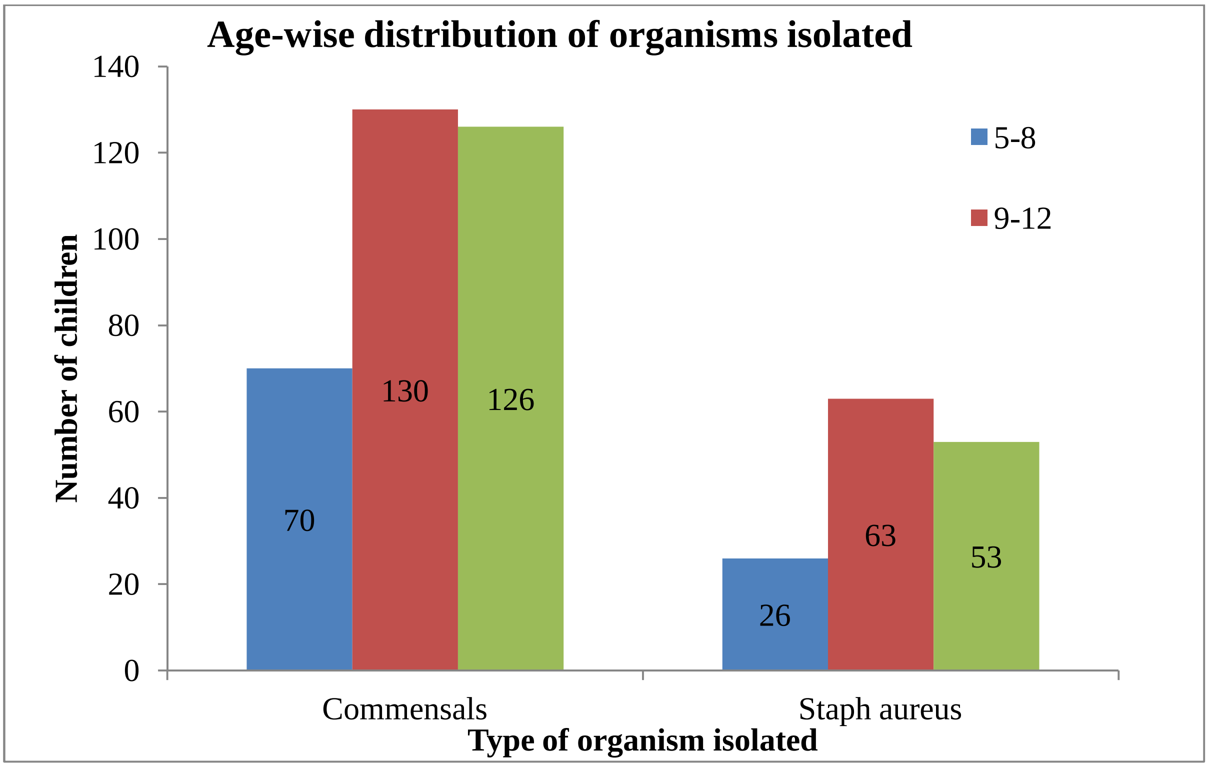


Table 6 depicts the school-wise distribution of *S. aureus* isolated in the nasal specimen of study participants. Out of the 15 schools selected, the range of prevalence was from 19.1% to 50%. Eight schools had prevalence of *Staphylococcus aureus* less than or equal to 30.3%, the overall prevalence obtained in the study, while the rest 7 schools had prevalence more than 30.3%.

Table 6 – Distribution of *Staph aureus* in nasal specimen of children of various schools

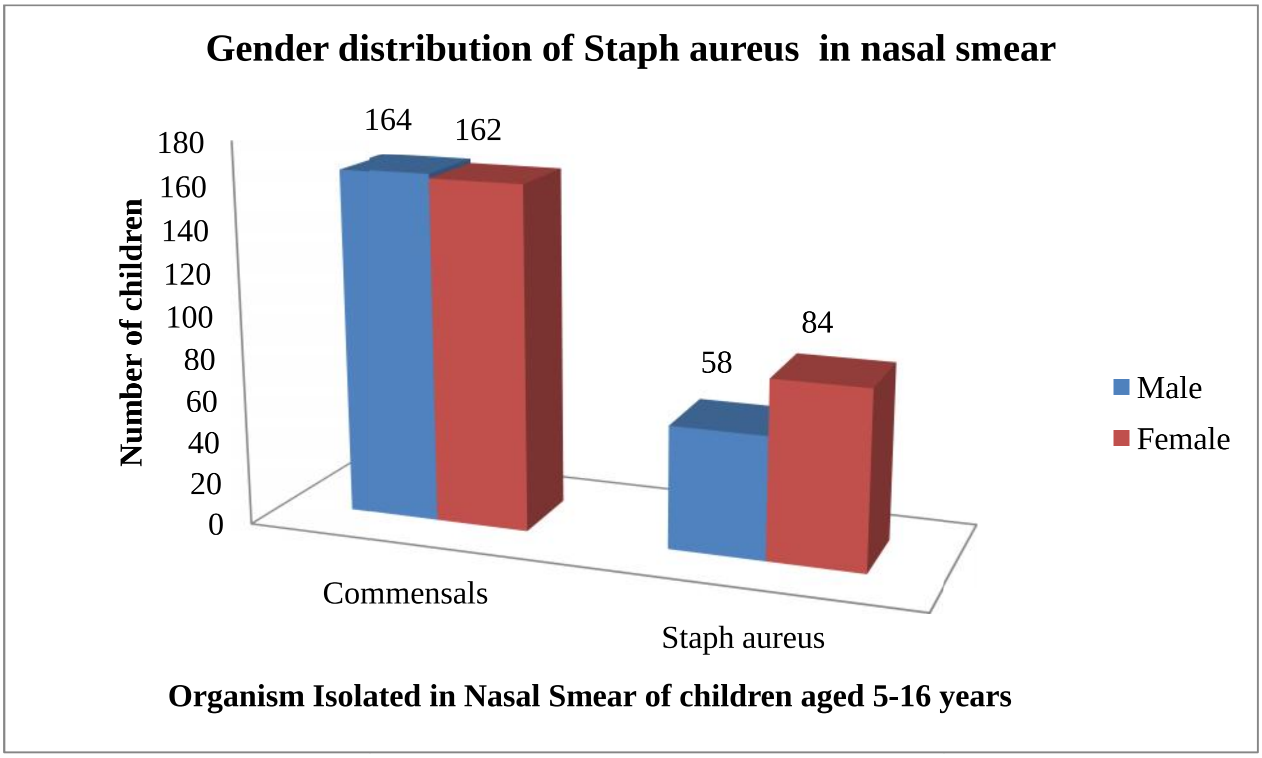
S No	Total students	Students selected	Staph aureus isolated	Prevalence of Staph aureus (%)
1	450	32	9	28.1
2	505	32	8	25.0
3	989	35	17	48.6
4	1399	105	21	20.0
5	544	35	15	42.9
6	357	64	18	28.1
7	295	21	4	19.0
8	284	20	5	25.0
9	268	21	7	33.3
10	132	9	2	22.2
11	100	8	4	50.0
12	234	18	7	38.9
13	274	19	4	21.1
14	499	34	15	44.1
15	695	15	6	40.0
TOTAL	**7025**	**468**	**142**	**30.3**

Graph 5 depicts the comparative histograms of commensals and staph aureus isolated across the three age-groups of 5-8, 9-12, and 13-17 years.



Graph 6 graphically represents the sex-wise distribution of *Staph aureus* among the study participants.

Graph 6 – Gender Distribution of *Staph aureus* and commensals



Objective 2- Prevalence of CA-MRSA

We then proceeded to find the distribution of different types of *Staph aureus* in the sample. Out of the total 142 samples which tested positive for *Staph aureus*, 113 were sensitive to Cefoxitin and were thus classified as ***Methicilin-sensitive Staph aureus (MSSA)***. There were 46 male and 67 female participants whose nasal smears was having MSSA.

Therefore, ***MRSA*** was isolated from the nasal smear of 29 study participants leading to the overall prevalence of ***MRSA*** to be 6.2%. The prevalence was reported to be higher among female students at 6.9 % than among male students at 5.4%. These results are depicted in **Table 7** and **Graph 7**

Table 7 – Prevalence of Nasal Carriage of *MRSA* (n=468)

S No	Parameter	Male	Female	Total
1	Total samples tested	222 (47.4%)	246 (52.6%)	468 (100%)
2	Prevalence of MSSA	46 (%)	67 (%)	113 (24%)
3	Prevalence of MRSA	12 (5.4%)	17 (6.9%)	**29 (6.2%)**

The result of distribution of types of ***Staph aureus*** based on their Methicilin sensitivity pattern is depicted in **Graph 7**

Graph 7– Prevalence of Nasal Carriage of *MRSA* (n=468)

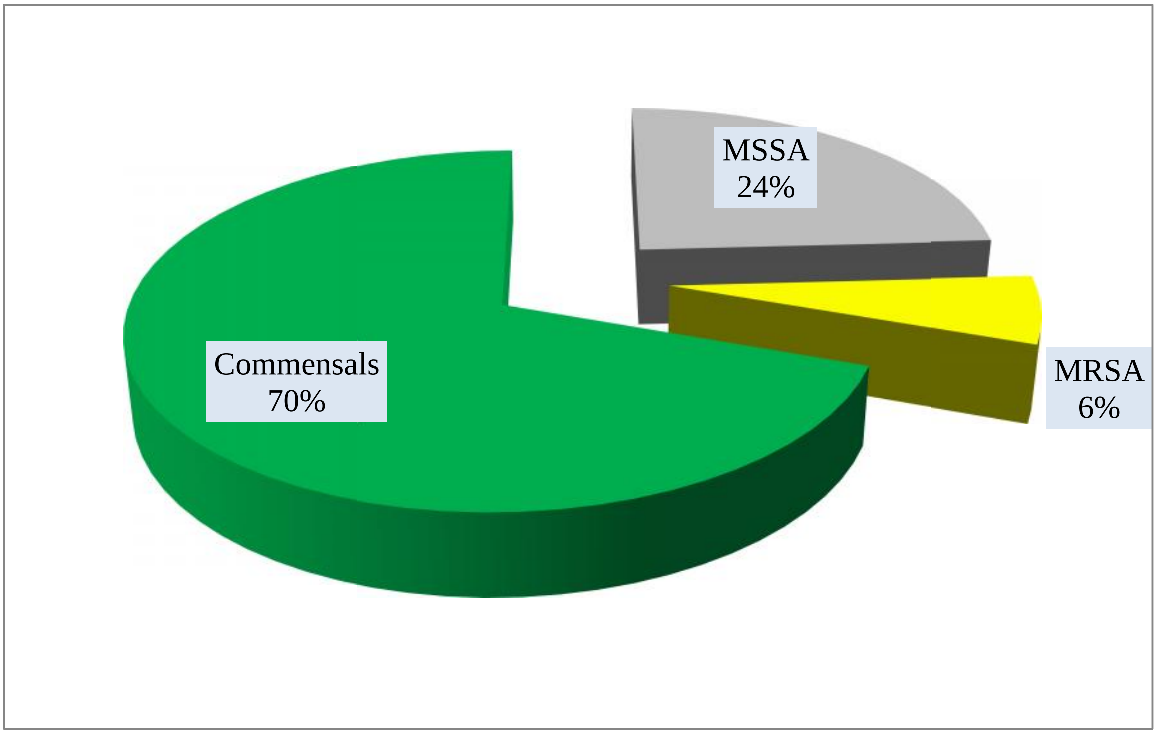


Table 8- Distribution of MRSA among different school

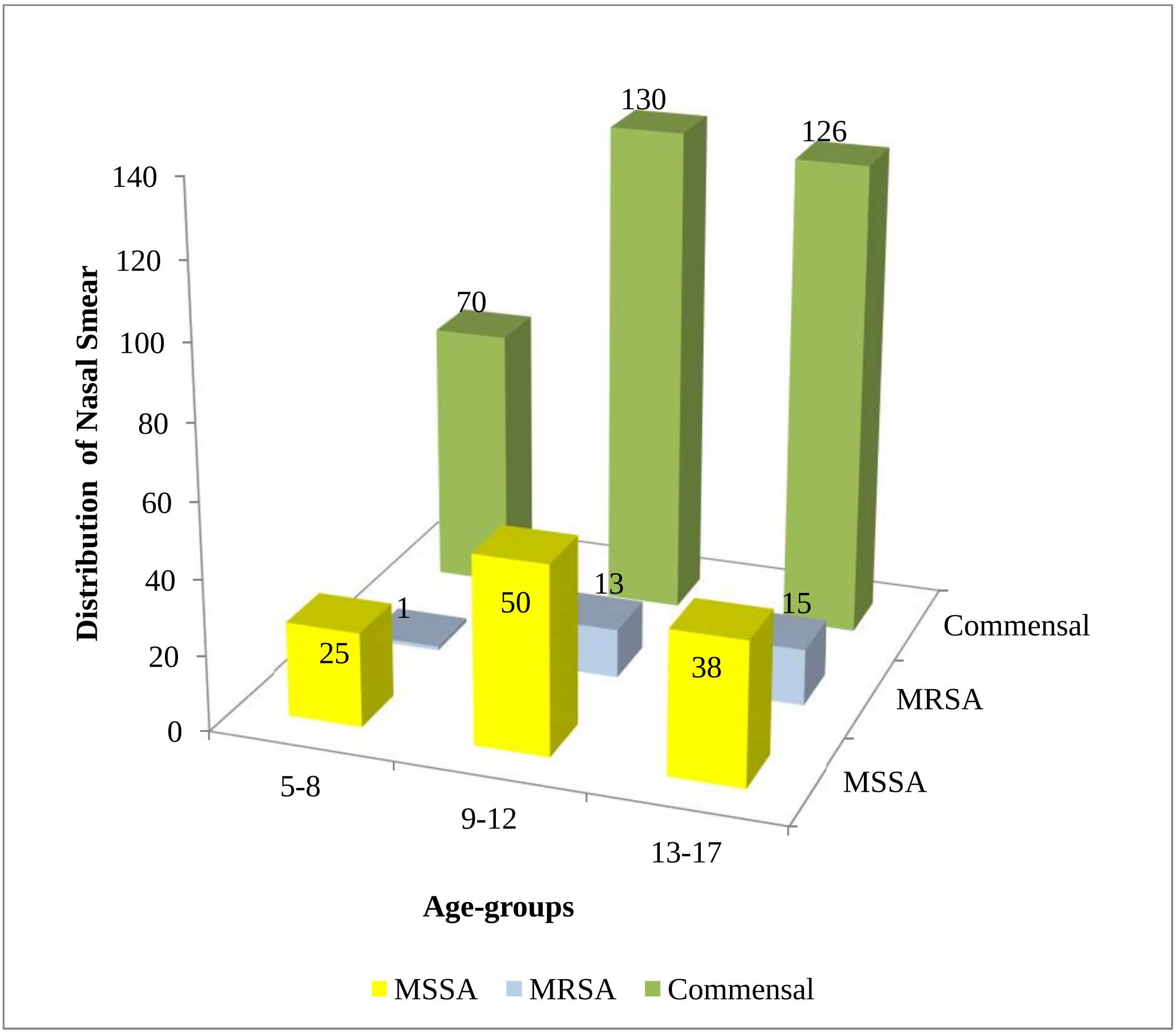
Table 10 depicts the school-wise distribution of MRSA isolated in the nasal specimen of study participants. Out of the 15 schools studied, eight schools didn't have any child with MRSA in their nasal smears. Five schools had children with MRSA prevalence higher than 6.2%, the overall MRSA prevalence. Only two schools had prevalence less than 6.2%, the overall MRSA prevalence in the study.

Table 8 – Distribution of MRSA in nasal specimen of children of various schools

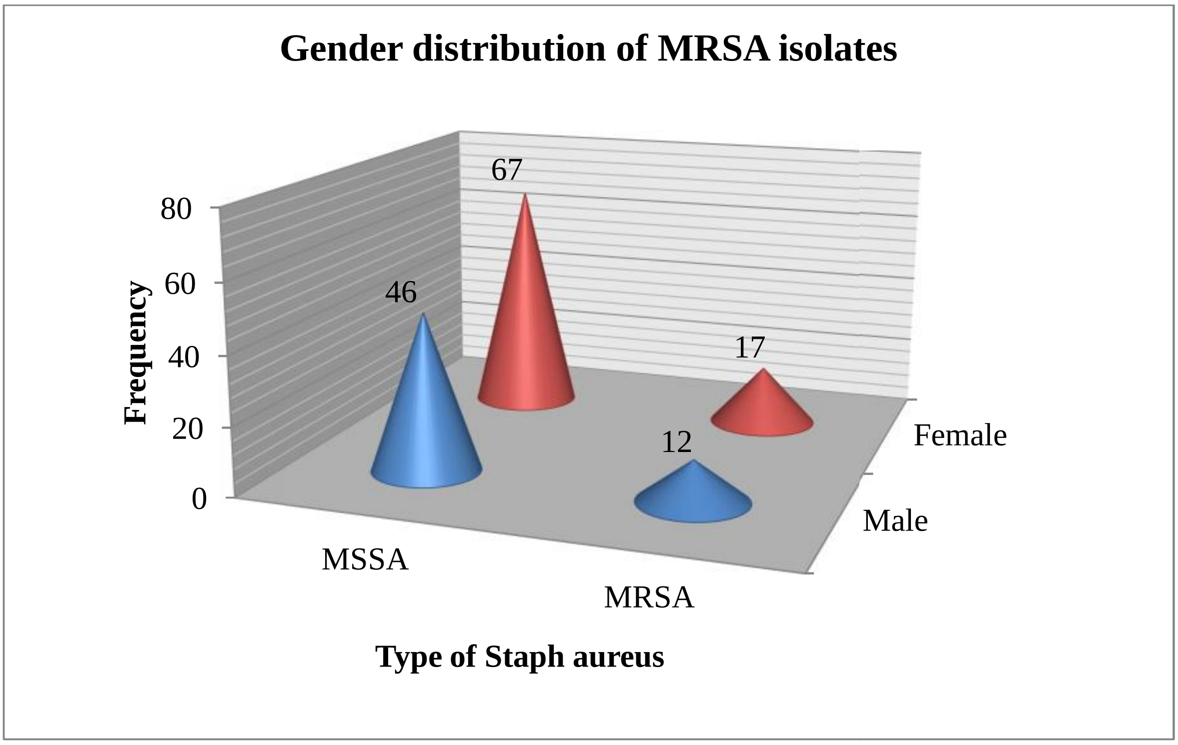
S No	Total students	Students selected	MRSA isolated	Prevalence of MRSA (%)
1	450	32	0	0
2	505	32	4	12.5
3	989	35	10	28.6
4	1399	105	3	2.9
5	544	35	2	5.7
6	357	64	5	7.8
7	295	21	0	0
8	284	20	0	0
9	268	21	0	0
10	132	9	0	0
11	100	8	0	0
12	234	18	0	0
13	274	19	2	10.5
14	499	34	3	8.8
15	695	15	0	0
TOTAL	**7025**	**468**	**29**	**6.2**

Graph 8- Prevalence of MSSA, MRSA and commensals is represented across the three age-groups of 5-8 years, 9-12 years and 13-17 years.

Graph 8– Distribution of *MRSA* across different age-groups (n=468)



Graph 9 depicts the gender distribution of MRSA isolated among the study participants.



Antibiotic sensitivity testing

Next the result of antibiotic sensitivity pattern of the Staph aureus isolates was explored. As evident in **Table 9**, majority of the isolates (97.9%) were resistant to Penicillin. A surprising feature was presence of Linezolid resistance among 7 isolates (4.9%). Amoxyclav and Clindamycin also had good sensitivity among the isolates.

Table 9– Antibiotic Sensitivity Pattern of *S aureus* isolated in study samples (n= 142)

S No	Antibiotic tested	Sensitive (%)	Resistant (%)
1	Penicillin	3 (2.1)	**139 (97.9)**
2	Erythromycin	95 (66.9)	**47 (33.1)**
3	Clindamycin	135 (95.1)	7 (4.9)
4	Cotrimoxazole	114 (80.3)	28 (19.7)
5	Cefoxitin	113 (79.6)	29 (20.4)
6	Amoxyclav	140 (98.6)	2 (1.4)
7	Linezolid	135 (95.1)	7 (4.9)
8	Ciprofloxacin	74 (52.1)	**68 (47.9)**

Graph 10 represents the sensitivity pattern of the *S aureus* isolates from the nasal smear of study participants.

Graph 10– Antibiotic Sensitivity Pattern of *S aureus* isolated in study samples

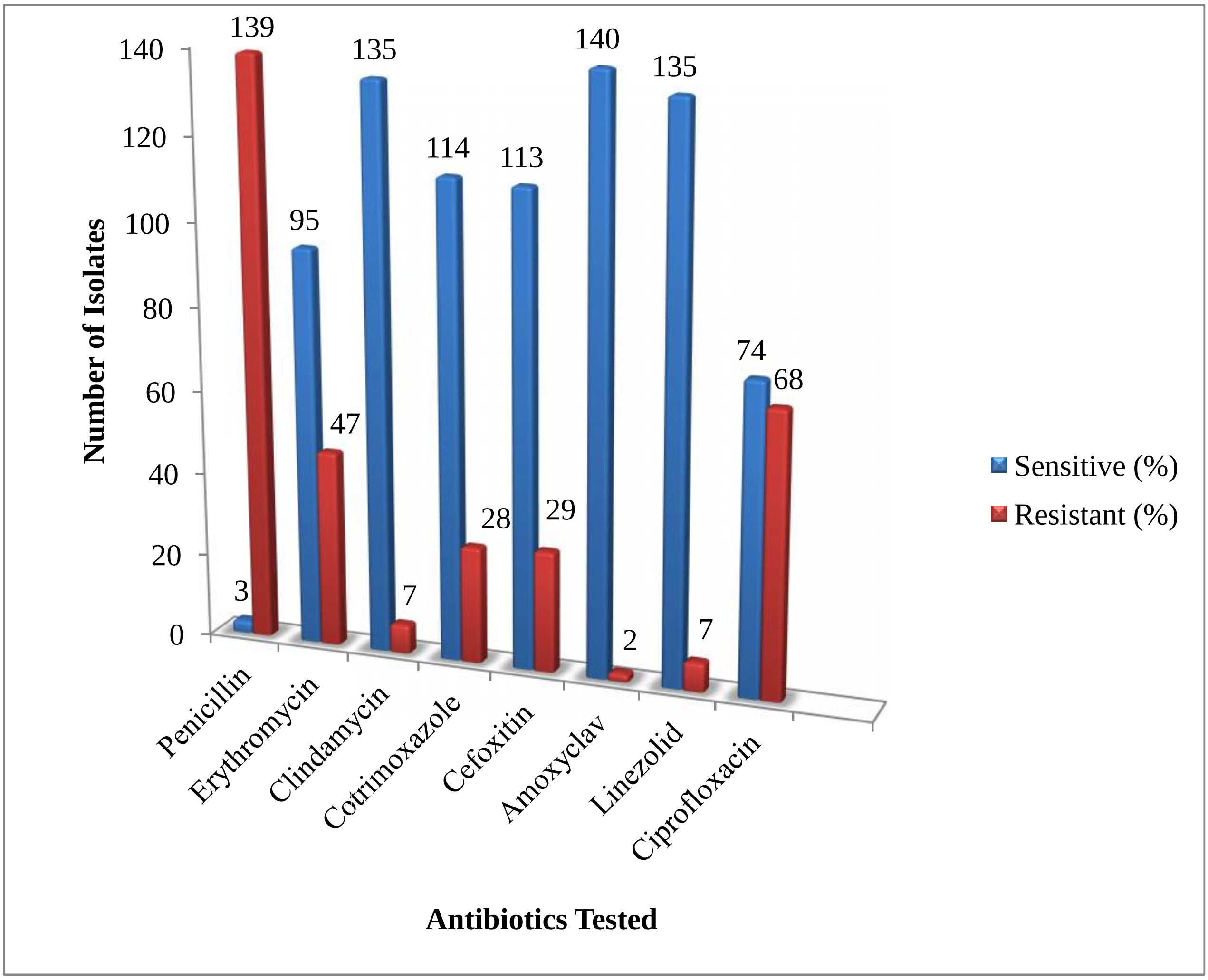
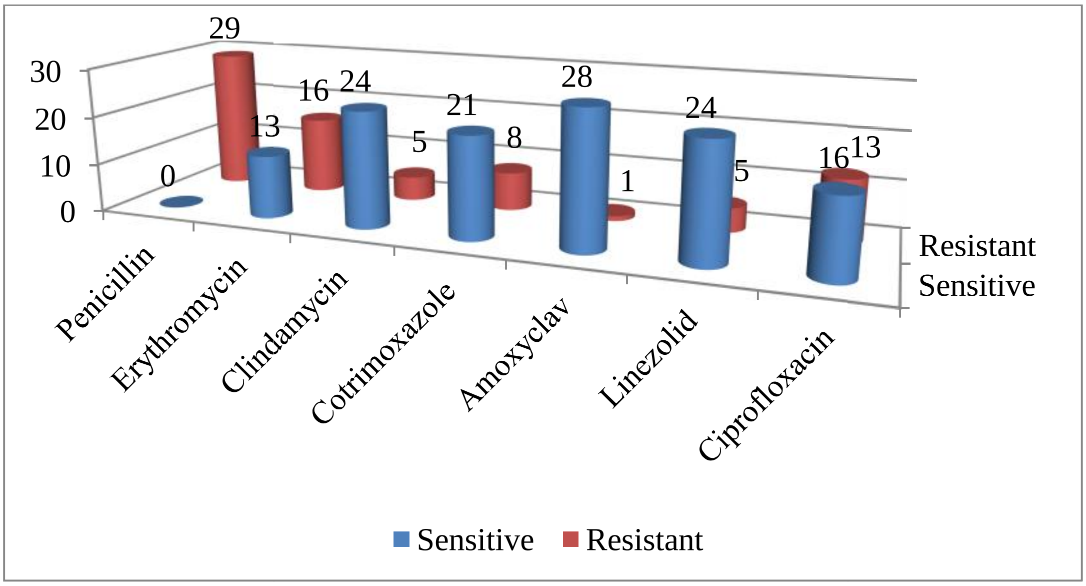


Table 10 represents the antibiotic sensitivity pattern of MRSA isolates from the study participants. As is evident, all the specimen were resistant to Penicillin. The MRSA isolates are getting resistant to erythromycin and ciprofloxacin too.

Table 10– Antibiotic sensitivity pattern of MRSA isolates of the study participants (n=29)

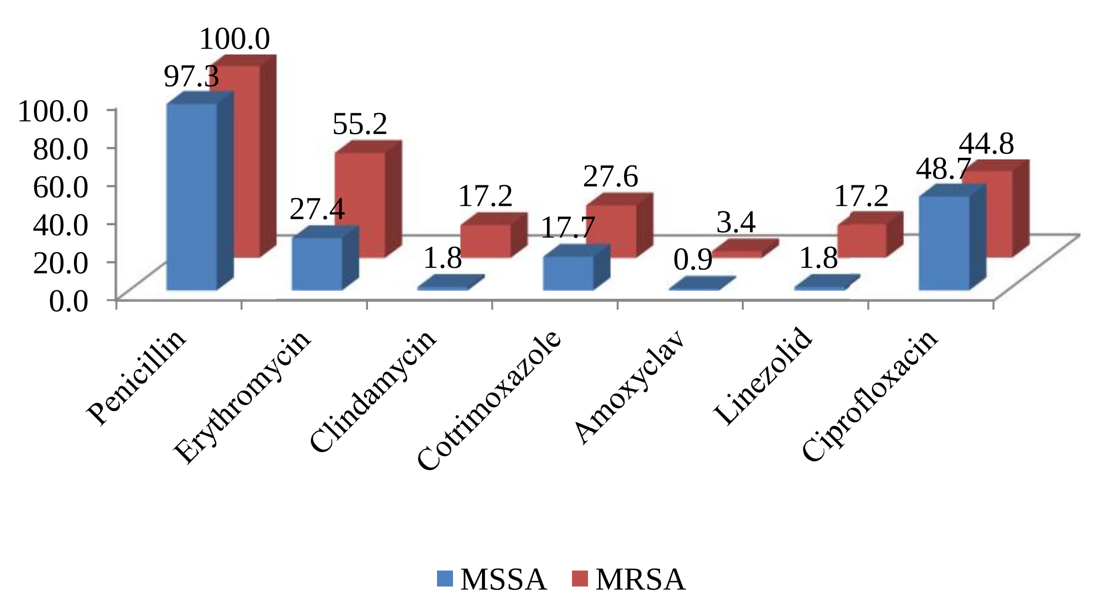
S No	Antibiotic tested	Sensitive (%)	Resistant (%)
1	Penicillin	0	29 (100)
2	Erythromycin	13 (44.8)	**16 (55.2)**
3	Clindamycin	24 (82.8)	5 (17.2)
4	Cotrimoxazole	21 (72.4)	8 (27.6)
5	Amoxyclav	28 (96.6)	1 (3.4)
6	Linezolid	24 (82.8)	5 (17.2)
7	Ciprofloxacin	16 (55.2)	**13 (44.8)**

Graph 11 – Antibiotic Sensitivity Pattern of MRSA isolated in study sample



Next, we compared the percentage resistance pattern between MSSA and MRSA isolates across the seven antibiotics used for testing sensitivity. The results are depicted in **Graph 12:**

Graph 12:
Comparison of percentage resistance between MSSA & MRSA



We then proceeded to explore the relationship of nasal carriage of *Staph aureus* as well as *MRSA* with various socio-demographic characteristics obtained from the study participants. We explored the association of presence of *Staph aureus* with age and sex of the participants.

We used chi square test to find the association between presence of *Staph aureus* in nasal specimen and sex of the participant. The X-square value obtained was 3.1821 and the p-value was 0.07445 which was not found to be significant at the 95% confidence level.

Table 11 – Association of various socio-demographic characteristics with *S.aureus* nasal colonisation

S No	Parameter	S aureus	Commensals	p-value
1	Mean age	11.2	11.2	0.89
2	Sex			
	Male	58	164	
	Female	84	162	0.075
3	Mean Family Size	5.07	4.9	0.08

Next, we explored the association of MRSA with various socio-demographic characteristics. The **Table 12** depicts the distribution of various socio-demographic factors across MRSA positive and negative study participants. Age was found to be statistically significantly distributed across MRSA positive and negative participants. The p-value at 95% confidence limit was 0.002 for mean age with

Table 12 – Association of various socio-demographic characteristics with presence of MRSA

S No	Parameter	MRSA+	MRSA-	p-value
1	Mean age	12.3	11.1	**0.002**
2	Sex			
	Male	12	210	0.63
	Female	17	229	
3	Mean Family Size	5.1	4.9	0.26

DISCUSSION

The present study from South India was conducted among healthy school children of age 5-16 years in community setting in which the total of 468 children were enrolled to determine the prevalence of nasal carriage of S. aureus and CA-MRSA.

Our study reports that 30% (142/468) of healthy school children in this geographical area are carrying or colonized with *S. aureus* in the anterior nares asymptomatically. This finding is consistent with reports of various other similar studies conducted in India and worldwide. The prevalence and incidence of S. aureus nasal carriage vary according to the population studied. The results of studies on nasal carriage as determined in cross-sectional surveys are shown in Table 1.

Study	**Place**	**S. aureus carriage rate**
Present	Belagavi, India	30.3%
Shetty V et.al(15)	Mangalore, India	25%
Fomda et. al(1)	Kashmir, India	27.92%
Govindan S. et. al(9)	Udupi, India	29.3%
Dey et. al(83)	Ujjain, India	35%

Studies from Taiwan and the U.S. have documented prevalence of nasal carriage of S. aureus among children ranging from 16% - 23% respectively (84,85).

In contrast, Chatterjee, SS et al., studied 489 school children aged 5-15 years by PCR and found nasal colonization of S. aureus in 256 (52.5%) of children, which is much higher compared to our study and other reports (10). The comparatively higher prevalence rate may be attributed to the characteristics of the study population, although other factors (e.g., sampling and culture techniques) may have played a contributory role.

Since there is regional variation in the colonisation of *S. aureus*, the overall prevalence of S. aureus colonization among healthy population has been estimated ranging from 20-40% worldwide.

The low prevalence of MRSA isolates, 29 (6.2%) in this study was found to be consistent with studies from the U.S., India, Turkey and Taiwan, reporting low rates of MRSA colonization among healthy children in the community ranging from 0.3% to 7.8% (85,10,86,84).

Study	**Place**	**CA-MRSA reported**
Present	Belagavi, India	6.2%
Shetty V et.al(15)	Mangalore, India	3%
Fomda et. al(1)	Kashmir, India	1.83%
Govindan S. et. al(9)	Udupi, India	1.1%
Chatterjee et.al(10)	Chandigarh, India	3.89%

In contrast, other studies from India and other countries have documented higher rates of MRSA colonization ranging from 13.2% to 22% (83,84,87,88). Dey et.al studied about 1000 preschool children of age group 1-6 years in Ujjain, India and reported the high prevalence of 29% of MRSA colonization.(83)

In one study from the U.S., the nasal colonization rate of MRSA among healthy children increased from 0.8% in 2001 to 9.2% in 2004 (89,90).

The differences in the carriage rate of S. aureus (and the percentages of MRSA isolates) from different regions suggesting geographical differences in the prevalence of MRSA colonization.

The prevalence of colonization with *S. aureus* has previously been shown to be age dependent (91–93). In our study, the prevalence varied across different age groups, with the lower prevalence in the 5-8 years of age group and being higher in 9-12 years of age group when compared to children of age group 13-16 years but it was not found statistically significant($p>0.05$).

In the present study, other socio-demographic factors like gender, type of house and family size was also studied for its association with the carriage of S. aureus . None of these were found statistically significant($p>0.05$) to be associated as a risk factors for colonization. Statistically significant risk factors for colonization included children below 6 y of age and members belonging to joint families. School going children were shown to have significantly higher prevalence of carriage. The finding is consistent with the fact that large family size with 10 or more members had higher carriage prevalence as compared to families with less than or equal to 4 members. This might be due to poor hygiene and overcrowding.

A similar study conducted by chattergee et.al has reported that there is no significant difference in CA-MRSA colonization rates between rural, urban and slum children(10).

Exposure to health- care facilities has been reported to cause increased carriage.our study excluding the same factor and others like antibiotic usage or any current infection, yielded a carriage rate of 6.2%.

Few studies has reported the asoociation of carriage rate of just 2.8% with an outbreak of CA-MRSA infections in American football players, rugby players and in a pediatric hospital resulting in death of children.(94–96). If we consider this low prevalence rate as the source of outbreak, then it can be considered as the alarming colonization rate of CA-MRSA in this community. CA-MRSA carriage rate in our study is reported comparatively higher (6.2%) than other studies conducted. It might be attributed to the favouring climatic condition of this geographical area, poor ventilation and overcrowding in few schools which were included in this study.

Asymptomatic colonization can persist for months to years.(91,95,97), however, the potential for outbreaks of disease and spread of the pathogen to different niches like hospital can cause havoc.(98)

In our study, the S. aureus isolates exhibited resistance to multiple classes of antibiotics including Cotriamoxazole resistance (19.7%), ciprofloxacin (47.9%), erythromycin resistance (33.1%), clindamycin resistance (4.9%), amoxyclav(1.4%), and linezolid(4.9%). Here, increasing resistance to linezolid is the worrisome fact to be drawn. No other studies have reported this rate of linezolid resistance in the community so far.

Other studies have also found similar results (99,100). In a recent study from Ujjain, MSSA isolates were found to be resistant to many classes of antibiotics including ampicillin (90%), amoxicillin clavulanate(54%), Cotriamoxazole(49%), ciprofloxacin (23%) and erythromycin (11%); of the erythromycin resistant strains of MSSA 15% were clindamycin inducible (101). A study from Portugal found that among the 36 S. aureus, only 11.5% of isolates were susceptible to all antibiotics tested; a higher non-susceptibility rate (88.5%) to penicillin was detected, which is similar to our finding of penicillin resistance(97.9%) (102). This may be due to fact that penicillin is most commonly prescribed as the first drug of choice. Compared to our study, a comparatively lower rate of resistance was found in the study conducted by Oguzkaya-Artan M, et al., where erythromycin resistance was noted in 6 of the 36 isolates (16.7 %) and clindamycin resistance was present in 3 of the 36 isolates.

High rate of resistance to Ciprofloxacin and Erythromycin in our setting is a concern since these antibiotics are routinely used to treat common infections due to *S. aureus* in the outpatient setting and often prescribed to hospitalized patients upon discharge to complete a course of outpatient therapy. Judicious antimicrobial use should be implemented by the physicians in this setting given the increasing prevalence of drug resistant microbes.

LIMITATIONS OF THE STUDY

Our study has several limitations. First, the sample size was relatively small. Second, we did not investigate the colonization at other body sites (such as, axillae, pharynx, and rectum) thereby underestimating the true prevalence in our study population. We elected to sample the nasal cavity due to easy of collection, adherence and consistency with other studies; in addition, studies have reported a relatively high sensitivity (~66%) of nose swabs in detecting MRSA carriage (103). Third, this observational, cross-sectional study was performed at a single location involving relatively healthy children, limiting the generalization of our results throughout the country. Fourth, we did not study the persistence of *S.aureus* nasal colonization, which would warrant a community based cohort design with repeated sampling of children over a period of time. Finally, we did not perform molecular typing of the strains due to lack of funding.

CONCLUSION

Our study reports the high prevalence of *S. aureus* nasal colonization(30.3%) in the children of 5-16 year age group and an alarming rate (6.2%) of community acquired methicillin resistant *S. aureus* (CA-MRSA) nasal colonization in the community.

This finding is of great concern as carriage of *S. aureus* in the nose appears to play a key role in the epidemiology and pathogenesis of infection. Strategies to interrupt transmission of *S. aureus* by elimination of nasal carriage and, thereby, preventing subsequent infection should be implemented. Continuing surveillance is needed to more accurately assess the prevalence, geographic distribution and epidemiology of community acquired infections.

The results emphasise the need to improve personal hygiene and discourage antibiotics abuse so as to prevent the return of the consequences of pre-antibiotic era. Judicious use of antibiotics, strengthening school health program and imparting health education regarding personal hygiene are the simple but effective measures to contain MRSA strains among Indian school children.

This study has demonstrated the baseline colonization rate and continued surveillance of this population is necessary to assess the ongoing risk posed by *S. aureus* to this community. The high rate of antibiotic resistance to frequently used antibiotics like Ciprofloxacin and Erythromycin is a major concern warranting continued surveillance and antimicrobial stewardship programs to promote judicious use of antimicrobials in the hospital and ambulatory settings

SUMMARY

- The study was conducted among children of age group 5-16 years from 15 different schools of urban Belgaum (Belagavi) located under Ramnagar Urban Health Centre(UHC) of KLE University's Jawahar Lal Nehru Medical College (JNMC)
- Out of 564 school children approached, 468 children were enrolled for the study. The paired nasal swabs were collected each from anterior nares of both the nostrils and processed for isolation and identification of S.aureus and CA-MRSA at the Department of Microbiology, Jawaharlal Nehru Medical College, Karnataka Lingayat Education University(KLEU), Belagavi
- Highest number of participants(41.23%) belonged to 9-12 years age-group.
- Most of the partcipants were 246 (52.6%) as compared to male students i.e; 222 (47.4%).
- The overall prevalence of nasal carriage of *S aureus* was found to be 30.3%.
- The prevalence of S.aureus colonization among male students was found to be 26.1% and that among female 34.1%.
- Higher *S,aureus* colonization rate was found to be among children of age group 9-12 years.
- CA-MRSA was isolated from the nasal smear of 29 study participants leading to the overall prevalence of CA-MRSA to be 6.2%.
- The prevalence of CA-MRSA nasal carriage was reported to be higher among female students at 6.9 % than among male students at 5.4%.
- Children of age group 13-16 years were found to be colonized with CA-MRSA at higher rate compared to others.

- None of the studied socio-demographic factors like age, sex, type of house and number of family members found to be statistically significant associated with nasal colonization of either *S. aureus* or MRSA.
- Antimicrobial susceptibility testing for S.aureus isolates reported higher rate of resistnance against Penicillin, Erythromycin and Ciprofloxacin. Amoxyclav and Clindamycin had good sensitivity among the isolates.
- CA-MRSA isolates also reported the same resistance pattern as *S. aureus*
- MRSA strains showed higher rate of resistance than MSSA isolates against all the antibiotic tested.

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ANNEXURE -I – ETHICAL CLEARANCE LETTER

K.L.E.UNIVERSITY'S

JAWAHARLAL NEHRU MEDICAL COLLEGE,

NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)

(Accredited 'A' Grade by NAAC)

Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu

Phone: (+ 91-(0)831 Office : 2471350
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 386 **Date: 19/11/2015**

To,

PG student in Microbiology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"PREVALENCE OF NASAL CARRIAGE OF STAPHYLOCOCCUS AUREUS AMONG SCHOOL CHILDREN OF AN URBAN AREA – ONE YEAR STUDY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Ganga Pilli)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE -II – LETTER FOR PHAGE TYPING

KLE University's
Jawaharlal Nehru Medical College, Belgaum-10
Department of Microbiology

Dr. Sumati Hogade MD. Professor of Microbiology
College Office: 0831-2471350, Depart: 0831-2473777 Extn: 4068 Fax No 0831-2470759
Email: principal@jnmc.edu. Web Site:www.jnmc.edu.

Ref.No.MDC/Micro/ Date:- 26.10.2017

To,

Prof & HoD (Dept of Microbiology) and
Incharge (National Staphylococcus Phage Typing Centre)
Maulana Azad Medical College, New Delhi

Subject: Request to permit phage typing of *Staphylococcus aureus* isolates collected from community-based study as part of MD (Microbiology) thesis

(Through proper channel)

Respected Sir,

I am final year post-graduate student in Microbiology at Jawahar Lal Nehru Medical College (JNMC), KLE University Belagavi (Belgaum). Under guidance of Dept of Microbiology, I conducted my thesis on the topic "*Prevalence of nasal carriage of Staphylococcus aureus among school children of an urban area –A one year study*". As the result of this thesis I was able to isolate 142 non-duplicate isolates of *Staphylococcus aureus.*

The isolated samples of *Staphylococcus aureus* need to be classified through phage typing. Your institute is the apex centre in India for phage typing for Staphylococcus aureus. I request you to permit us in getting all the 142 isolates of *S aureus* phage-typed so that I will be able to document their classification and share with the scientific community. Please guide me in the procedure. I would be grateful for this kind gesture.

Thanking you in advance with sincere regards,

Prof. & Head of Microbiology,
J.N.Medical College,
KLE University Belagavi.

Post graduate student
MD (Microbiology)
JNMC, KLEU
Belagavi, Karnataka

Contact No: 9448866944, Email ID:sumatihogade@yahoo.co.in

ANNEXURE -III – CONSENT AND ASSENT FORM

PARTICIPANTS INFORMATION SHEET

Dear Parents,

Kindly read this document which provides information on a medical research to be conducted.

TITLE:Prevalence of nasal carriage of *Staphylococcus aureus* among school children of an urban area- one year study.

Study Investigator **Dr.**

Department of Microbiology,

Jawaharlal Nehru Medical College,

KLE University, Belagavi– 590 010

Guide **Dr.** **,**MD**(**Microbiology)

Professor,

Department of Microbiology,

J. N. Medical College, KLE University,

Belagavi – 590 010.

Brief Introduction of study

A research study will be conducted titled as above among school children of age 5-15yrs.Your child is requested to participate in this study.

Staphylococcus aureus is one of the common causes for variety of infections ranging from mild skin and soft tissue infection to life threatening infection. It is commonly present in anterior nares of nose, most commonly in children without any symptoms.

Due to its multi-drug resistance property, its very difficult to treat, once the infection occurs. So it is very important to know well in advance if your child is carrying this bacteria or not.

Hence this study will help you to know the same and if your child is carrying this bacteria ,it can be treated well in time before it causes infection.

What will happen if your child participates in this study?

If you agree for participation of your child in this study, you will have to answer some questions to the best of your knowledge and will also have to give consent by signing the consent form which is attached with this letter. Only after your permission, sample will be collected from your child by rotating a cotton swab in anterior part of the nose.

Risk

This procedure will not cause any pain or harm to your child.

Benefits

The participation of your child in this study will be beneficial to your child and to the community you are living in, as treatment can be given in time and spread of this bacteria can be prevented .

Voluntary participation

Your participation in this research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with Jawaharlal Nehru Medical College. If you decide to participate you are free to withdraw at any time.

Privacy and confidentiality:

The only people to know that your child is a research subject are members of the research team. No information about your child or report of the study will be disclosed to others without your written permission.

Whom should you contact if you have any question about the study?

In case you have any questions about your rights as a participant, you can contact

Dr Ganga S. Pilli, Chairman, KLE university's JNMC Institutional Ethical Committee for Human Subjects Research**, Ph.-0831-2471350 Ext: 4052** at J.N. Medical College, Belagavi.

Authorization to publish results:

When the results of research are published or discussed, in a conference no information will be displaced that would disclose your identity. Any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Financial incentives for participation:

You will not receive money or have to pay/offer any gifts for participating in the research. You will not be reimbursed for expenses.

CONSENT STATEMENT

I, the undersigned ____have been explained in my vernacular language about the study and participation of my child in the study is with my consent. If I want, I can withdraw my child from the study at any time. Also I have been given enough time to clear my doubts and rights as study participant.

Signature or left thumb impression

Participants Name _______________________

Parent's Name ________________________signature_____________

Witness Name __________________________signature______________

Experimenters Name ___________________signature______________

Date :

Place:

ASSENT FORM

I, the undersigned ____have been explained in my vernacular language about the study and my participation in this study is voluntary. If I want, I can withdraw from the study at any time. Also I have been given enough time to clear my doubts and rights as study participant.

Signature or left thumb impression

Participant's Name ____________________ signature______________

Witness Name ________________________signature_______________

Experimenters Name __________________ signature_______________

Date :

Place:

ಭಾಗವಹಿಸುವವರುಮಾಹಿತಿನಮೂನೆ

ಆತ್ಮೀಯಪೋಷಕರೆ,

ನಡೆಸುವವೈದ್ಯಕೀಯಸಂಶೋಧನಾಮಾಹಿತಿಯನ್ನುಒದಗಿಸುವಈದಾಖಲೆಯನ್ನುದಯವಿಟ್ಟುಓದಿರಿ.

ಶೀರ್ಷಿಕೆ: ನಗರಪ್ರದೇಶಶಾಲೆಯಮಕ್ಕಳಲ್ಲಿಸ್ಟ್ಯಾಫಿಲೋಕೊಕಸ್ಔರೆಸ್ಮೂಗಿನಸಾರೋಟುಹರಡಿಕೆ-ಒಂದುವರ್ಷಅಧ್ಯಯನ

ಅಧ್ಯಯನದಪರೀಕ್ಷಕರು

ಮೈಕ್ರೋಬಯಾಲಜಿಇಲಾಖೆ,
ಜವಾಹರಲಾಲ್ನೆಹರೂಮೆಡಿಕಲ್ಕಾಲೇಜ್,
ಕೆಎಲ್ಇವಿಶ್ವವಿದ್ಯಾಲಯ, ಬೆಳಗಾವಿ - ೫೯೦೦೧೦

ಮಾರ್ಗದರ್ಶಕರು

ಪ್ರಾಧ್ಯಾಪಕರು ,
ಮೈಕ್ರೋಬಯಾಲಜಿಇಲಾಖೆ,
ಜವಾಹರಲಾಲ್ನೆಹರೂಮೆಡಿಕಲ್ಕಾಲೇಜ್,
ಕೆಎಲ್ಇವಿಶ್ವವಿದ್ಯಾಲಯ, ಬೆಳಗಾವಿ - ೫೯೦೦೧೦

ಅಧ್ಯಯನದಕಿರುಪರಿಚಯ

ಮೇಲ್ಕಂಡಹೆಸರಿನಒಂದುಸಂಶೋಧನಾಅಧ್ಯಯನೆಯನ್ನ೫-೧೫ವರ್ಷದಶಾಲಾಮಕ್ಕಳಲ್ಲಿನಡೆಸಲಾಗುವುದು,ಇದರಲ್ಲಿನಿಮ್ಮಮಗುಭಾಗವಹಿಸುವಂತೆಕೊರುತ್ತೇನೆ.ಸ್ಟ್ಯಾಫಿಲೋಕೊಕಸ್ಔರೆಸ್ವಿವಿಧಸೋಂಕುಗಳನ್ನಉಂಟುಮಾಡುವಸಾಮಾನ್ಯಕಾರಣಗಳಲ್ಲಿಒಂದು.ಇದುಸೌಮ್ಯಚರ್ಮಮತ್ತುಸೂಕ್ಷ್ಮವಾದಅಂಗಾಂಶಗಳಸೋಂಕಿನಿಂದಹಿಡಿದುಜೀವಕ್ಕೆತೊಂದರೆಯನ್ನುಂಟುಮಾಡುವಸೋಂಕುಗಳನ್ನಉಂಟುಮಾಡಬಹುದು.ಇದುಯಾವುದೇಲಕ್ಷಣಗಳುಇಲ್ಲದೆಮಕ್ಕಳಲ್ಲಿಸಾಮಾನ್ಯವಾಗಿ, ಮೂಗಿನಮುಂಭಾಗದಹೊಳ್ಳೆಗಳಲ್ಲಿಸಾಮಾನ್ಯವಾಗಿಇರುತ್ತದೆ.ಅದರಬಹುಔಷಧನಿರೋಧಕಶಕ್ತಿಇಂದ,ಒಮ್ಮೆಸೋಂಕುಉಂಟಾದರೆ,ಅದರಚಿಕಿತ್ಸೆತುಂಬಾಕಷ್ಟ;ಆದ್ದರಿಂದನಿಮ್ಮಮಗುವಿನಲ್ಲಿಈಬ್ಯಾಕ್ಟೀರಿಯಾಇದಿಯೊಇಲ್ಲವೊಎಂಬುದನ್ನಮುಂಚಿತವಾಗಿತಿಳಿಯುವುದುಬಹಳಮುಖ್ಯ.ಆದ್ದರಿಂದಈಅಧ್ಯಯನದಲ್ಲಿನೀವುಇದನ್ನತಿಳಿಯಬಹುದುಮತ್ತುನಿಮ್ಮಮಗುವಿನಲ್ಲಿಈಬ್ಯಾಕ್ಟೀರಿಯಾಇದ್ದಲ್ಲಿಅದುಸೋಂಕುಉಂಟುಮಾಡುವಮೊದಲೇಉತ್ತಮಚಿಕಿತ್ಸೆನೀಡಲುಸಹಾಯವಾಗುತ್ತದೆ.

ನಿಮ್ಮಮಗುಈಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸಿದರೆಏನಾಗುತ್ತದೆ?

ನಿಮ್ಮಮಗುಭಾಗವಹಿಸುವುದಕ್ಕೆನೀವುಒಪ್ಪಿದಲ್ಲಿ,ಕೆಲವುಪ್ರಶ್ನೆಗಳಿಗೆನೀವುನಿಮಗೆಅರಿವಿರುವಷ್ಟನ್ನಉತ್ತರಿಸಬೇಕುಮತ್ತುಇದರಜೊತೆಲಗತ್ತಿಸಿರುವಅನುಮತಿಪತ್ರಕ್ಕೆಸಹಿಹಾಕುವಮೂಲಕನಿಮ್ಮಅನುಮತಿಯನ್ನಸೂಚಿಸಬೇಕಾಗುತ್ತದೆ.ನಿಮ್ಮಸಂಮ್ಮತಿಯನಂತರವಷ್ಟೇ,ನಿಮ್ಮಮಗುವಿನಮೂಗಿನಮುಂಭಾಗದಲ್ಲಿಹತ್ತಿಹೀರುಮೆತ್ತೆತಿರುಗಿಸಿಮಾದರಿಯನ್ನಸಂಗ್ರಹಿಸಲಾಗುವುದು.

ಅಪಾಯ

ಈವಿಧಾನವುನಿಮ್ಮಮಗುವಿಗೆಯಾವುದೇನೋವುಅಥವಾಹಾನಿಉಂಟುಮಾಡುವುದಿಲ್ಲ.

ಲಾಭ

ನಿಮ್ಮಮಗುವಿನಭಾಗವಹಿಸುವಿಕೆಇಂದಸಮಯದಲ್ಲಿಚಿಕಿತ್ಸೆಯನ್ನನೀಡಬಹುದುಮತ್ತುಈಬ್ಯಾಕ್ಟೀರಿಯಾಹರಡುವುದನ್ನತಡೆಗಟ್ಟಬಹುದುಇದರಿಂದನಿಮ್ಮಮಗುವಿಗೆಮತ್ತುನೀವುವಾಸಿಸುತ್ತಿರವಸಮುದಾಯಕ್ಕೆಅನುಕೂಲವಾಗುತ್ತದೆ

ಸ್ವಯಂಪ್ರೇರಿತಭಾಗವಹಿಸುವಿಕೆ

ಸಂಶೋಧನೆಯಲ್ಲಿನಿಮ್ಮಭಾಗವಹಿಸುವಿಕೆವೈಯಕ್ತಿಕವಾಗಿದ್ದು.ಅಧ್ಯಯನದಲ್ಲಿಭಾಗವಹಿಸುವುದೊ ಇಲ್ಲವೋಎಂಬನಿಮ್ಮನಿರ್ಧಾರದಿಂದ;
ಜವಾಹರಲಾಲ್ನೆಹರೂವೈದ್ಯಕೀಯಕಾಲೇಜುಮತ್ತುನಿಮ್ಮನಡುವಿನಸಂಬಂಧದಮೇಲೆಯೂವುದೇಪರಿಣಾಮಬೀರುವುದಿಲ್ಲ. ನೀವುಭಾಗವಹಿಸಲುನಿರ್ಧರಿಸಿದರೆ ; ಯಾವುದೇಸಮಯದಲ್ಲಿನಿಮ್ಮ ನಿರ್ಧಾರವನ್ನು ಬದಲಿಸಲುನೀವು ಪೂರ್ಣ ಸ್ವತಂತ್ರರಾಗಿರುತ್ತೀರಿ.

ಗೌಪ್ಯತೆ:

ಸಂಶೋಧನೆಯತಂಡದವರನ್ನಹೊರತುಪಡಿಸಿಬೆರೆಯಾರಿಗೊನಿಮ್ಮಮಗುಸಂಶೋಧನೆಗೆಒಳಪಟ್ಟಿರುವುದುತಿಳಿದಿರುವುದಿಲ್ಲ,
ನಿಮ್ಮಲಿಖಿತಅನುಮತಿಯಿಲ್ಲದೆನಿಮ್ಮಮಗುವಿನಬಗ್ಗೆಅಥವಾಅದರಸಂಶೋಧನೆಯವರದಿಯಬಗ್ಗೆಇತರರಿಗೆಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ.

ನಿಮ್ಮಹಕ್ಕುಗಳಬಗ್ಗೆಯಾವುದೇಪ್ರಶ್ನೆಗಳನ್ನುಹೊಂದಿದ್ದರೆ, ನೀವುಸಂಪರ್ಕಿಸಬಹುದು

ಡಾಗಂಗಾಎಸ್ಪಿಳ್ಳೈ, ಪ್ರಾಧ್ಯಾಪಕರುಪೆಥಾಲಜಿಇಲಾಖೆಮತ್ತುಅಧ್ಯಕ್ಷರುಇನ್ಸ್ಟಿಟೊಷನಲ್ಎತಿಕ್ಸ್ಕಮಿಟಿ, ಜೆಎನ್ಎಮ್ಸಿ (ಕಚೇರಿ # 0831-2471350 ಎಕ್ಸ್ಟ: 4052)

ಫಲಿತಾಂಶಗಳುಪ್ರಕಟಿಸಲುಅಧಿಕಾರ:

ಸಂಶೋಧನೆಯಫಲಿತಾಂಶಗಳನ್ನಪ್ರಕಟಿಸಿದಾಗಅಥವಾಕಾನ್ಫರೆನ್ಸ್ ಳಲ್ಲಿಚರ್ಚಿಸಿದಾಗನಿಮ್ಮಗುರುತನ್ನುಬಹಿರಂಗಪಡಿಸಬಹುದಾದಯೊವುದೇವಿಷಯದಪ್ರಸ್ತಾಪಮಾಡಲಾಗುವುದಿಲ್ಲ.ಈಅಧ್ಯಯನಕ್ಕೆಸಂಬಂಧಪಟ್ಟಂತೆ,ನಿಮ್ಮಿಂದಪಡೆದಯಾವುದೇಮಾಹಿತಿನಿಮ್ಮಜೊತೆಗುರುತಿಸುವಂತಿದ್ದಲ್ಲಿಅದನ್ನುಕೂಡಗೌಪ್ಯವಾಗಿಡಲಾಗುವುದು.

ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಗಾಗಿನಹಣಕಾಸುಪ್ರೋತ್ಸಾಹ:

ಈ

ಸಂಶೋಧನೆಯಲ್ಲಿಭಾಗವಹಿಸಲುನಿಮಗೆನಾವುಯಾವುದೇಹಣಪಾವತಿಅಥವಾಉಡುಗೊರೆಯನ್ನನೀಡುವುದಿಲ್ಲಮತ್ತುನಿಮ್ಮಯಾವುದೇವೆಚ್ಚಗಳನ್ನನಾವುಭರಿಸುವುದಿಲ್ಲ.

<u>ಅನುಮತಿ</u>

ಈಕೆಳಗೆರುಜುಮಾಡಿರುವ____________________ನಾನಗೆನನ್ನದೇಶೀಯಭಾಷೆಯಲ್ಲಿಈಅಧ್ಯಯನದಬಗ್ಗೆವಿವರಿಸಲಾಗಿದೆಮತ್ತುಅಧ್ಯಯನದಲ್ಲಿನನ್ನಮಗುವಿನಭಾಗವಹಿಸುವಿಕೆಗೆನನ್ನಸಂಮ್ಮತಿಇದೆ. ನನ್ನಮಗುಬಯಸಿದರೆ, ಯಾವುದೇಸಮಯದಲ್ಲಿನನ್ನಮಗುವನ್ನಈಅಧ್ಯಯನದಿಂದಹಿಂದಕ್ಕೆಪಡೆಯಬಹುದು .ಹಾಗೆಯೇನಾನುಅಧ್ಯಯನಭಾಗಿಯಾಗಿನನ್ನಅನುಮಾನಗಳನ್ನಪರಿಹರಿಸಿಕೊಳ್ಳಲುಮತ್ತುಹಕ್ಕುಗಳಬಗ್ಗೆತಿಳಿದುಕೊಳ್ಳಲು ಸಾಕಷ್ಟುಸಮಯನೀಡಲಾಗಿದೆ.

ಸಹಿಅಥವಾಎಡಗೈಹೆಬ್ಬೆರಳುಮುದ್ರಣ.

ಭಾಗವಹಿಸುವವರಹೆಸರು ________________

ಪೋಷಕರಹೆಸರು ________________ **ಸಹಿ**____________

ಸಾಕ್ಷಿದಾರರಹೆಸರು ________________ **ಸಹಿ**____________

ಪ್ರಯೋಗಿಕರಹೆಸರು ________________ **ಸಹಿ**____________

ದಿನಾಂಕ: **ಸ್ಥಳ:**

ಒಪ್ಪಿಗೆಫಾರ್ಮ್

ನಾನುರುಜುಮಾಡಿರುವ____ಅಧ್ಯಯನದ ಬಗ್ಗೆನನ್ನದೇಶೀಯ ಭಾಷೆಯಲ್ಲಿವಿವರಿಸಲಾಗಿದೆಮತ್ತುಈ ಅಧ್ಯಯನದಲ್ಲಿನನ್ನ ಭಾಗವಹಿಸುವಿಕೆವೈಯಕ್ತಿಕವಾಗಿದ್ದು.ನಾನುಬಯಸಿದರೆ, ನಾನುಯಾವುದೇ ಸಮಯದಲ್ಲಿಅಧ್ಯಯನತನ್ನ ಪಾಲನ್ನು ಹಿಂಪಡೆಯಬಹುದಾಗಿದೆ.ಹಾಗೆಯೇ ನಾನುಅಧ್ಯಯನಭಾಗಿಯಾಗಿನನ್ನ ಅನುಮಾನಗಳನ್ನುಮತ್ತುಹಕ್ಕುಗಳತೆರವುಗೊಳಿಸಲು ಸಾಕಷ್ಟುಸಮಯನೀಡಲಾಗಿದೆ.

ಸಹಿಅಥವಾಎಡಹೆಬ್ಬೆಟ್ಟಿನ ಗುರುತು

ಭಾಗಿಯಹೆಸರು ಸಹಿ_

ವಿಟ್ನೆಸ್ಹೆಸರು ಸಹಿ_

ಪ್ರಯೋಗಹೆಸರು ಸಹಿ

ದಿನಾಂಕ:

ಸ್ಥಳ:

ANNEXURE -IV – QUESTIONNAIRE

QUESTIONNAIRE FOR COLLECTING DATA

Name:

LAB. NO :

Age:

DOC :

Sex :

Std :

Roll no.

Address :

Name of school –

Exclusion criteria-

- H/O any skin and soft tissue infection - yes/no
- H/O hospitalisation within last 12 months - yes/no
- H/O recent exposure to health care

facilities due to any other reason - yes/no

- H/O intake of any antibiotic currently or

within last 7 days - yes/no

- Socio-demographic details-
  - No. of members in the family currently living together - ……….
  - Type of house – Brick / Mud-thatch

ಪ್ರಶ್ನಾವಳಿ

ಮಗುವಿನಹೆಸರು-

ವಯಸ್ಸು –

ಲಿಂಗ–

ವಿಳಾಸ-

ಶಾಲೆಯಹೆಸರು-

ನಿಮ್ಮಮಗುವಿಗೆಯಾವುದಾದರುಚರ್ಮದಸೋಂಕುಇದೆಯೇ?ಹೌದು/ ಇಲ್ಲ (ಹೌದುಎಂದಾದರೆಯಾವಾಗಿನಿಂದ?)

ಕಳೆದ 12 ತಿಂಗಳಲ್ಲಿನಿಮ್ಮಮಗುವನ್ನಯಾವುದಾದರುಆಸ್ಪತ್ರೆಗೆಸೇರಿಸಿದ್ದಿರಾಅಥವಾಆಸ್ಪತ್ರೆಗೆ / ಕ್ಲಿನಿಕ್ಭೇಟಿನೀಡಿದ್ದೀರಾ?
-ಹೌದು/ ಇಲ್ಲ

ನಿಮ್ಮಮಗುಈಗಯಾವುದೇಪ್ರತಿಜೀವಕಸೇವಿಸುತ್ತಿದೆಯಾಅಥವಾಕಳೆದ 7 ದಿನಗಳಲ್ಲಿಸೇವಿಸಿದೆಯಾ ?ಹೌದು/ ಇಲ್ಲ

ನಿಮ್ಮಕುಟುಂಬದಲ್ಲಿಒಟ್ಟಿಗೆವಾಸಿಸುತ್ತಿರುವಸದಸ್ಯರಸಂಖ್ಯೆ-

ಮನೆಯಪ್ರಕಾರ - ಇಟ್ಟಿಗೆ / ಮಣ್ಣಿನಹುಲ್ಲಿನ -

ದಿನಾಂಕ:

ಸ್ಥಾನ:

ANNEXURE -V – PROFORMA

Lab Findings

	On Mannitol salt agar	On Sheep Blood agar
Colony Morphology		

Confirmation by-

1) Gram' stain-
2) Catalase test-
3) Coagulase test-

   Slide coagulase test-

   Tube coagulase test-
4) Urease test-

Antibiotic sensitivity testing-

Antibiotics	Sensitive	Resistant
1) Penicillin (10unit)		
2) Amoxyclav (20/10 µg)		
3) Erythromycin (15 µg)		
4) Clindamycin (2 µg)		
5) Cotrimoxazole (25µg)		
6)Ciprofloxacin (5 µg)		
7) Linezolid (30µg)		
8) Cefoxitin(30 µg)		

ANNEXURE -VI –KEY TO MASTER CHART

S No	Variable Name	Full Form	Details of Codes
1	id	Identification No of participants	
2	school	School Name	
3	age	Age of Student	
4	sex	Sex of Student	1 - Male, 2 - Female
5	std	Class of Student	actual class studying now in digits (eg 3 for student studying in class third)
6	famsize	Number of members in the family currently living together	Actual number of members
7	housetype	Type of House	Brick - 1, Mud-thatch - 2, Not known - 99
8	msa	Colony Morphology on Mannitol Salt Agar	Sensitive - 0, Resistant - 1, Intermediate
9	sba	Colony Morphology on Sheep Blood Agar	No colony - 0, beta-hemolysis with small circular white colony - 1, Beta-hemolysis with minute colony - 2, Others - 88
10	gram	Gram Stain Confirmation	Gram positive Cocci in cluster - 1, Gram positive micrococci - 2, Gram Positive Bacilli - 3
11	catalase	Result of Catalase Test	Positive - 1, Negative - 0, Not applicable - 99
12	coag_slide	Result of Slide Coagulase Test	Positive - 1, Negative - 0, Not applicable - 99
13	coag_tube	Result of Tube Coagulase Test	Positive - 1, Negative - 0, Not applicable - 99
14	urease	Result of Urease Test	Positive - 1, Negative - 0, Not applicable - 99
15	P	Antibiotic Sensitivity - Penicillin	Sensitive - 0, Resistant - 1, Not applicable - 99
16	E	Antibiotic Sensitivity - Erythromycin	Sensitive - 0, Resistant - 1, Not applicable - 99
17	CD	Antibiotic Sensitivity - Clindamycin	Sensitive - 0, Resistant - 1, Not applicable - 99
18	COT	Antibiotic Sensitivity - Cotrimoxazole	Sensitive - 0, Resistant - 1, Not applicable - 99
19	CX	Antibiotic Sensitivity - Cefoxitin	Sensitive - 0, Resistant - 1, Not applicable - 99
20	CIP	Antiobiotic Sensitivity - Ciprofloxacin	Sensitive - 0, Resistant - 1, Not applicable - 99
21	AMC	Antibiotic Sensitivity - Amoxyclav	Sensitive - 0, Resistant - 1, Not applicable - 99
22	LZ	Antibiotic Sensitivity - Linezolid	Sensitive - 0, Resistant - 1, Not applicable - 99
23	outcome	Outcome of nasal smear	MSSA - 1, MRSA - 2, Commensals - 3
24	sa	Staphylococcus aureus present in nasal smear	Yes - 1, No - 0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
1	Govt Kannada Medium School	7	1	1	6	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
2	Govt Kannada Medium School	8	2	2	5	2	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	1	1
3	Govt Kannada Medium School	9	1	3	9	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1
4	Govt Kannada Medium School	12	2	4	5	2	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
5	Govt Kannada Medium School	10	2	5	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
6	Govt Kannada Medium School	11	2	6	6	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
7	Govt Kannada Medium School	14	2	7	2	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
8	Govt Kannada Medium School	13	1	7	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
9	Govt Kannada Medium School	6	2	1	4	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
10	Govt Kannada Medium School	7	2	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
11	Govt Kannada Medium School	9	2	3	4	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
12	Govt Kannada Medium School	9	2	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
13	Govt Kannada Medium School	10	1	5	6	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	1	1
14	Govt Kannada Medium School	11	2	5	5	2	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
15	Govt Kannada Medium School	11	2	7	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
16	Govt Kannada Medium School	11	2	7	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
17	Govt Kannada Medium School	12	2	7	6	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
18	Govt PHQ Kannada School	7	1	1	4	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
19	Govt PHQ Kannada School	8	1	2	8	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
20	Govt PHQ Kannada School	9	1	3	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
21	Govt PHQ Kannada School	9	2	3	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
22	Govt PHQ Kannada School	9	2	3	6	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
23	Govt PHQ Kannada School	10	1	4	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
24	Govt PHQ Kannada School	11	1	4	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
25	Govt PHQ Kannada School	11	2	4	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
26	Govt PHQ Kannada School	11	1	5	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
27	Govt PHQ Kannada School	11	1	5	4	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
28	Govt PHQ Kannada School	11	2	5	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
29	Govt PHQ Kannada School	12	1	6	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
30	Govt PHQ Kannada School	12	2	6	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
31	Govt PHQ Kannada School	12	1	6	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
32	Govt PHQ Kannada School	12	2	6	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
33	Govt PHQ Kannada School	13	1	7	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
34	Govt PHQ Kannada School	13	1	7	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
35	Govt PHQ Kannada School	13	2	7	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
36	Govt PHQ Kannada School	13	2	7	6	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
37	Govt PHQ Kannada School	13	2	7	4	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
38	Govt PHQ Kannada School	13	2	7	6	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
39	Govt Urdu School	6	1	1	5	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
40	Govt Urdu School	6	2	1	6	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
41	Govt Urdu School	7	2	1	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
42	Govt Urdu School	7	1	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
43	Govt Urdu School	7	2	2	3	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
44	Govt Urdu School	8	2	3	6	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
45	Govt Urdu School	8	2	3	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
46	Govt Urdu School	9	1	4	4	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
47	Govt Urdu School	9	2	4	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
48	Govt Urdu School	9	2	4	5	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
49	Govt Urdu School	10	1	5	3	1	0	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
50	Govt Urdu School	10	2	5	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
51	Govt Urdu School	10	2	5	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
52	Govt Urdu School	11	1	6	4	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1
53	Govt Urdu School	11	2	6	3	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
54	Govt Urdu School	11	2	6	4	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
55	Govt Urdu School	12	1	7	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
56	Govt Urdu School	12	2	7	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
57	Madani Higher Primary School	10	1	5	4	1	1	1	1	1	1	1	1	0	1	0	1	0	0	0	0	1	1
58	Madani Higher Primary School	11	1	5	5	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
59	Madani Higher Primary School	11	2	5	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
60	Madani Higher Primary School	12	2	5	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
61	Madani Higher Primary School	12	2	5	13	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
62	Madani Higher Primary School	12	2	5	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
63	Madani Higher Primary School	12	2	5	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
64	Madani Higher Primary School	11	2	5	5	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
65	Madani Higher Primary School	12	2	5	7	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
66	Madani Higher Primary School	10	2	5	6	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
67	Madani Higher Primary School	12	1	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
68	Madani Higher Primary School	14	1	6	4	2	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1
69	Madani Higher Primary School	12	2	6	6	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
70	Madani Higher Primary School	12	2	6	6	2	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
71	Madani Higher Primary School	12	2	6	6	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	2	1
72	Madani Higher Primary School	12	2	6	7	1	2	8	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
73	Madani Higher Primary School	12	2	6	6	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	2	1
74	Madani Higher Primary School	12	2	6	6	1	0	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
75	Madani Higher Primary School	12	2	6	4	1	0	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
76	Madani Higher Primary School	12	1	7	6	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	2	1
77	Madani Higher Primary School	12	1	7	5	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	2	1
78	Madani Higher Primary School	13	1	7	8	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
79	Madani Higher Primary School	13	1	7	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
80	Madani Higher Primary School	13	2	7	6	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	2	1
81	Madani Higher Primary School	12	2	7	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
82	Madani Higher Primary School	13	2	7	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
83	Madani Higher Primary School	13	2	7	5	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
84	Madani Higher Primary School	13	2	7	5	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	2	1

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
85	Madani Higher Primary School	14	1	8	5	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	2	1
86	Madani Higher Primary School	14	1	8	6	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
87	Madani Higher Primary School	14	2	8	7	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
88	Madani Higher Primary School	14	2	8	6	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	2	1
89	Madani Higher Primary School	14	2	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
90	Madani Higher Primary School	14	2	8	6	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	2	1
91	Madani Higher Primary School	14	2	8	4	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	2	1
92	Mahila Vidyalaya Marathi High School	11	2	6	4	1	1	1	1	1	1	1	1	0	0	0	0	0	1	0	0	1	1
93	Mahila Vidyalaya Marathi High School	11	2	6	5	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
94	Mahila Vidyalaya Marathi High School	11	2	7	5	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	0	2	1
95	Mahila Vidyalaya Marathi High School	12	2	7	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
96	Mahila Vidyalaya Marathi High School	12	2	7	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
97	Mahila Vidyalaya Marathi High School	12	2	7	4	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
98	Mahila Vidyalaya Marathi High School	13	2	8	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
99	Mahila Vidyalaya Marathi High School	13	2	8	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
100	Mahila Vidyalaya Marathi High School	13	2	8	4	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
101	Mahila Vidyalaya Marathi High School	13	2	8	6	1	0	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
102	Mahila Vidyalaya Marathi High School	13	2	8	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
103	Mahila Vidyalaya Marathi High School	13	2	8	4	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	2	1
104	Mahila Vidyalaya Marathi High School	13	2	8	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
105	Mahila Vidyalaya Marathi High School	14	2	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
106	Mahila Vidyalaya Marathi High School	14	2	9	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
107	Mahila Vidyalaya Marathi High School	14	2	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
108	Mahila Vidyalaya Marathi High School	14	2	9	4	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
109	Mahila Vidyalaya Marathi High School	14	2	9	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
110	Mahila Vidyalaya Marathi High School	14	2	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
111	Mahila Vidyalaya Marathi High School	14	2	9	4	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
112	Mahila Vidyalaya Marathi High School	14	2	9	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
113	Mahila Vidyalaya Marathi High School	14	2	9	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
114	Mahila Vidyalaya Marathi High School	14	2	9	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
115	Mahila Vidyalaya Marathi High School	14	2	9	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
116	Mahila Vidyalaya Marathi High School	15	2	10	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
117	Mahila Vidyalaya Marathi High School	15	2	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
118	Mahila Vidyalaya Marathi High School	15	2	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
119	Mahila Vidyalaya Marathi High School	15	2	10	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
120	Mahila Vidyalaya Marathi High School	15	2	10	6	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
121	Mahila Vidyalaya Marathi High School	15	2	10	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
122	Mahila Vidyalaya Marathi High School	15	2	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
123	Mahila Vidyalaya Marathi High School	15	2	10	4	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
124	Mahila Vidyalaya Marathi High School	16	2	10	7	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
125	Mahila Vidyalaya Marathi High School	16	2	10	11	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
126	Mahila Vidyalaya Marathi High School	15	2	10	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
127	KLE BS Hanchial school	6	1	1	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
128	KLE BS Hanchial school	6	1	1	3	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
129	KLE BS Hanchial school	6	1	1	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
130	KLE BS Hanchial school	7	1	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
131	KLE BS Hanchial school	7	2	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
132	KLE BS Hanchial school	8	1	3	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
133	KLE BS Hanchial school	9	2	3	5	1	0	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
134	KLE BS Hanchial school	8	1	3	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
135	KLE BS Hanchial school	8	1	4	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
136	KLE BS Hanchial school	9	2	4	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
137	KLE BS Hanchial school	10	2	5	6	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
138	KLE BS Hanchial school	10	1	5	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
139	KLE BS Hanchial school	10	1	5	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
140	KLE BS Hanchial school	11	1	6	4	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
141	KLE BS Hanchial school	11	1	6	4	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
142	KLE BS Hanchial school	11	2	6	6	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
143	KLE BS Hanchial school	12	1	7	4	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
144	KLE BS Hanchial school	13	2	7	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
145	KLE BS Hanchial school	13	1	7	4	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
146	KLE BS Hanchial school	12	2	7	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
147	NPET English Medium High School	6	2	1	5	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
148	NPET English Medium High School	6	1	1	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
149	NPET English Medium High School	6	1	1	5	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1
150	NPET English Medium High School	7	2	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
151	NPET English Medium High School	9	2	2	6	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
152	NPET English Medium High School	8	1	2	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
153	NPET English Medium High School	7	1	2	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
154	NPET English Medium High School	9	1	3	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
155	NPET English Medium High School	9	1	3	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
156	NPET English Medium High School	9	2	3	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
157	NPET English Medium High School	10	2	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
158	NPET English Medium High School	10	2	4	5	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	2	1
159	NPET English Medium High School	10	2	5	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
160	NPET English Medium High School	11	2	5	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
161	NPET English Medium High School	10	1	5	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
162	NPET English Medium High School	10	1	5	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
163	NPET English Medium High School	10	2	5	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
164	NPET English Medium High School	11	1	6	5	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1
165	NPET English Medium High School	11	1	6	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
166	NPET English Medium High School	11	1	6	4	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
167	NPET English Medium High School	12	1	7	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
168	NPET English Medium High School	12	1	7	4	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
169	NPET English Medium High School	12	2	7	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
170	NPET English Medium High School	13	2	8	4	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0	2	1
171	NPET English Medium High School	13	2	8	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
172	NPET English Medium High School	13	1	8	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
173	NPET English Medium High School	13	2	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
174	NPET English Medium High School	14	2	9	6	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
175	NPET English Medium High School	13	1	9	6	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
176	NPET English Medium High School	13	2	9	6	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
177	NPET English Medium High School	15	1	10	6	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
178	NPET English Medium High School	15	1	10	6	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	2	1
179	NPET English Medium High School	16	1	10	7	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
180	NPET English Medium High School	15	2	10	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
181	KLE GA School	11	2	6	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
182	KLE GA School	11	1	6	5	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	2	1
183	KLE GA School	12	1	6	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	1
184	KLE GA School	12	2	7	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	1	1	1	1
185	KLE GA School	13	2	7	4	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	2	1
186	KLE GA School	12	1	7	6	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
187	KLE GA School	12	1	7	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
188	KLE GA School	12	1	7	5	1	1	1	1	1	1	1	1	1	0	0	0	1	1	0	1	2	1
189	Sharman Kannada Primary School	6	1	1	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
190	Sharman Kannada Primary School	6	1	1	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
191	Sharman Kannada Primary School	6	2	1	4	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
192	Sharman Kannada Primary School	7	1	2	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
193	Sharman Kannada Primary School	7	1	2	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
194	Sharman Kannada Primary School	7	1	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
195	Sharman Kannada Primary School	7	2	2	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
196	Sharman Kannada Primary School	8	1	3	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
197	Sharman Kannada Primary School	8	1	3	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
198	Sharman Kannada Primary School	8	1	3	5	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
199	Sharman Kannada Primary School	8	2	3	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
200	Sharman Kannada Primary School	9	1	4	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
201	Sharman Kannada Primary School	9	1	4	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
202	Sharman Kannada Primary School	9	1	4	5	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0	2	1
203	Sharman Kannada Primary School	9	2	4	5	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	2	1
204	Sharman Kannada Primary School	10	1	5	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
205	Sharman Kannada Primary School	10	2	5	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
206	Sharman Kannada Primary School	10	2	5	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
207	Sharman Kannada Primary School	10	1	5	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
208	Sharman Kannada Primary School	10	1	5	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
209	Sharman Kannada Primary School	10	2	5	5	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1
210	Sharman Kannada Primary School	12	1	6	4	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	2	1
211	Sharman Kannada Primary School	11	1	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
212	Sharman Kannada Primary School	12	2	6	4	1	0	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
213	Sharman Kannada Primary School	12	1	6	5	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	2	1
214	Sharman Kannada Primary School	12	1	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
215	Sharman Kannada Primary School	12	2	6	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
216	Sharman Kannada Primary School	13	1	6	5	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
217	Sharman Kannada Primary School	12	1	7	7	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
218	Sharman Kannada Primary School	12	2	7	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
219	Sharman Kannada Primary School	12	1	7	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
220	Sharman Kannada Primary School	12	2	7	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
221	Sharman Kannada High School	13	1	8	6	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	2	1
222	Sharman Kannada High School	12	2	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
223	Sharman Kannada High School	13	2	8	5	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
224	Sharman Kannada High School	12	2	8	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
225	Sharman Kannada High School	13	1	8	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
226	Sharman Kannada High School	13	1	8	6	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
227	Sharman Kannada High School	13	2	8	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
228	Sharman Kannada High School	14	2	9	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
229	Sharman Kannada High School	14	2	9	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
230	Sharman Kannada High School	14	2	9	4	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1
231	Sharman Kannada High School	14	2	9	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
232	Sharman Kannada High School	14	2	9	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
233	Sharman Kannada High School	15	1	10	6	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
234	Sharman Kannada High School	15	1	10	6	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
235	Sharman Kannada High School	15	1	10	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
236	Sharman Kannada High School	15	1	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
237	Sharman Kannada High School	15	1	10	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
238	Sharman Kannada High School	15	1	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
239	Sharman Kannada High School	15	1	10	5	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	2	1
240	Mahila Vidyalaya English Medium School	6	1	1	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
241	Mahila Vidyalaya English Medium School	6	1	1	4	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
242	Mahila Vidyalaya English Medium School	6	1	1	4	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
243	Mahila Vidyalaya English Medium School	6	2	1	4	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
244	Mahila Vidyalaya English Medium School	6	1	1	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
245	Mahila Vidyalaya English Medium School	6	1	1	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
246	Mahila Vidyalaya English Medium School	6	2	1	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
247	Mahila Vidyalaya English Medium School	6	2	1	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
248	Mahila Vidyalaya English Medium School	6	1	1	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
249	Mahila Vidyalaya English Medium School	6	1	1	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
250	Mahila Vidyalaya English Medium School	6	2	1	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
251	Mahila Vidyalaya English Medium School	7	1	2	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
252	Mahila Vidyalaya English Medium School	7	1	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
253	Mahila Vidyalaya English Medium School	7	1	2	4	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
254	Mahila Vidyalaya English Medium School	7	2	2	4	1	1	1	1	1	1	1	1	1	1	1	0	1	0	0	1	2	1
255	Mahila Vidyalaya English Medium School	7	1	2	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
256	Mahila Vidyalaya English Medium School	7	1	2	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
257	Mahila Vidyalaya English Medium School	7	2	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
258	Mahila Vidyalaya English Medium School	7	2	2	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
259	Mahila Vidyalaya English Medium School	7	1	2	4	1	0	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
260	Mahila Vidyalaya English Medium School	7	1	2	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
261	Mahila Vidyalaya English Medium School	7	2	2	4	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1
262	Mahila Vidyalaya English Medium School	8	1	3	4	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1
263	Mahila Vidyalaya English Medium School	8	1	3	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
264	Mahila Vidyalaya English Medium School	9	1	3	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
265	Mahila Vidyalaya English Medium School	9	2	3	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
266	Mahila Vidyalaya English Medium School	8	1	3	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
267	Mahila Vidyalaya English Medium School	8	1	3	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
268	Mahila Vidyalaya English Medium School	8	2	3	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
269	Mahila Vidyalaya English Medium School	8	2	3	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
270	Mahila Vidyalaya English Medium School	9	1	3	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
271	Mahila Vidyalaya English Medium School	9	1	3	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
272	Mahila Vidyalaya English Medium School	8	2	3	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
273	Mahila Vidyalaya English Medium School	9	1	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
274	Mahila Vidyalaya English Medium School	10	1	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
275	Mahila Vidyalaya English Medium School	9	2	4	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
276	Mahila Vidyalaya English Medium School	9	2	4	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
277	Mahila Vidyalaya English Medium School	9	1	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
278	Mahila Vidyalaya English Medium School	10	1	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
279	Mahila Vidyalaya English Medium School	10	2	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
280	Mahila Vidyalaya English Medium School	10	2	4	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
281	Mahila Vidyalaya English Medium School	9	1	4	5	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
282	Mahila Vidyalaya English Medium School	9	2	4	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
283	Mahila Vidyalaya English Medium School	9	2	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
284	Mahila Vidyalaya English Medium School	10	1	5	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
285	Mahila Vidyalaya English Medium School	10	1	5	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
286	Mahila Vidyalaya English Medium School	10	2	5	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
287	Mahila Vidyalaya English Medium School	11	2	5	5	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1
288	Mahila Vidyalaya English Medium School	11	1	5	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
289	Mahila Vidyalaya English Medium School	10	1	5	5	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1
290	Mahila Vidyalaya English Medium School	10	2	5	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
291	Mahila Vidyalaya English Medium School	10	2	5	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
292	Mahila Vidyalaya English Medium School	11	1	5	5	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1
293	Mahila Vidyalaya English Medium School	11	1	5	6	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
294	Mahila Vidyalaya English Medium School	10	2	5	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
295	Mahila Vidyalaya English Medium School	11	1	6	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
296	Mahila Vidyalaya English Medium School	11	1	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
297	Mahila Vidyalaya English Medium School	11	2	6	4	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
298	Mahila Vidyalaya English Medium School	11	2	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
299	Mahila Vidyalaya English Medium School	11	1	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
300	Mahila Vidyalaya English Medium School	12	1	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
301	Mahila Vidyalaya English Medium School	11	2	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
302	Mahila Vidyalaya English Medium School	11	2	6	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
303	Mahila Vidyalaya English Medium School	12	1	6	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
304	Mahila Vidyalaya English Medium School	11	2	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
305	Mahila Vidyalaya English Medium School	11	2	6	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
306	Mahila Vidyalaya English Medium School	12	1	7	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
307	Mahila Vidyalaya English Medium School	13	1	7	6	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
308	Mahila Vidyalaya English Medium School	12	2	7	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
309	Mahila Vidyalaya English Medium School	12	2	7	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
310	Mahila Vidyalaya English Medium School	12	1	7	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
311	Mahila Vidyalaya English Medium School	12	2	7	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
312	Mahila Vidyalaya English Medium School	13	2	7	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
313	Mahila Vidyalaya English Medium School	13	1	7	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
314	Mahila Vidyalaya English Medium School	12	1	7	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
315	Mahila Vidyalaya English Medium School	12	2	7	5	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	2	1
316	Mahila Vidyalaya English Medium School	12	2	7	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
317	Mahila Vidyalaya English Medium School	13	1	8	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
318	Mahila Vidyalaya English Medium School	14	1	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
319	Mahila Vidyalaya English Medium School	13	2	8	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
320	Mahila Vidyalaya English Medium School	13	1	8	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
321	Mahila Vidyalaya English Medium School	13	1	8	5	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0	2	1
322	Mahila Vidyalaya English Medium School	13	2	8	6	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
323	Mahila Vidyalaya English Medium School	13	2	8	5	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
324	Mahila Vidyalaya English Medium School	13	1	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
325	Mahila Vidyalaya English Medium School	14	1	8	5	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	0	1	1
326	Mahila Vidyalaya English Medium School	14	2	8	4	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
327	Mahila Vidyalaya English Medium School	13	1	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
328	Mahila Vidyalaya English Medium School	14	1	9	5	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
329	Mahila Vidyalaya English Medium School	15	2	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
330	Mahila Vidyalaya English Medium School	15	1	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
331	Mahila Vidyalaya English Medium School	14	1	9	5	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1
332	Mahila Vidyalaya English Medium School	14	2	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
333	Mahila Vidyalaya English Medium School	14	2	9	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
334	Mahila Vidyalaya English Medium School	15	1	9	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
335	Mahila Vidyalaya English Medium School	15	1	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
336	Mahila Vidyalaya English Medium School	14	2	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
337	Mahila Vidyalaya English Medium School	16	1	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
338	Mahila Vidyalaya English Medium School	15	1	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
339	Mahila Vidyalaya English Medium School	16	2	10	5	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
340	Mahila Vidyalaya English Medium School	15	2	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
341	Mahila Vidyalaya English Medium School	15	1	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
342	Mahila Vidyalaya English Medium School	16	1	10	5	1	0	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
343	Mahila Vidyalaya English Medium School	16	2	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
344	Mahila Vidyalaya English Medium School	15	2	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
345	Mahila Vidyalaya Marathi Primary School	6	2	1	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
346	Mahila Vidyalaya Marathi Primary School	6	2	1	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
347	Mahila Vidyalaya Marathi Primary School	6	2	1	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
348	Mahila Vidyalaya Marathi Primary School	7	2	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
349	Mahila Vidyalaya Marathi Primary School	7	2	2	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
350	Mahila Vidyalaya Marathi Primary School	8	2	2	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
351	Mahila Vidyalaya Marathi Primary School	7	2	2	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
352	Mahila Vidyalaya Marathi Primary School	8	2	3	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
353	Mahila Vidyalaya Marathi Primary School	8	2	3	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
354	Mahila Vidyalaya Marathi Primary School	8	2	3	6	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
355	Mahila Vidyalaya Marathi Primary School	8	2	3	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
356	Mahila Vidyalaya Marathi Primary School	9	2	4	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
357	Mahila Vidyalaya Marathi Primary School	10	2	4	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
358	Mahila Vidyalaya Marathi Primary School	9	2	4	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
359	Mahila Vidyalaya Marathi Primary School	9	2	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
360	Mahila Vidyalaya Marathi Primary School	9	2	4	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
361	Mahila Vidyalaya Marathi Primary School	10	2	5	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
362	Mahila Vidyalaya Marathi Primary School	11	2	5	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
363	Mahila Vidyalaya Marathi Primary School	10	2	5	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
364	Mahila Vidyalaya Marathi Primary School	10	2	5	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
365	Mahila Vidyalaya Marathi Primary School	10	2	5	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
366	Sharman English Medium School	6	2	1	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
367	Sharman English Medium School	6	1	1	6	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1
368	Sharman English Medium School	6	2	1	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
369	Sharman English Medium School	6	2	1	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
370	Sharman English Medium School	7	2	2	4	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
371	Sharman English Medium School	7	1	2	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
372	Sharman English Medium School	8	2	3	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
373	Sharman English Medium School	8	2	3	5	1	0	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
374	Sharman English Medium School	12	2	3	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
375	Sharman English Medium School	9	1	4	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
376	Sharman English Medium School	10	1	4	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
377	Sharman English Medium School	10	1	4	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
378	Sharman English Medium School	10	1	5	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
379	Sharman English Medium School	10	1	5	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
380	Sharman English Medium School	10	1	5	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
381	Sharman English Medium School	11	1	6	6	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
382	Sharman English Medium School	12	1	6	6	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
383	Sharman English Medium School	11	1	6	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
384	Sharman English Medium School	11	1	6	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
385	Sharman English Medium School	12	2	7	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
386	Sharman English Medium School	12	1	7	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
387	Sharman English Medium School	12	1	7	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
388	Sharman English Medium School	12	2	7	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
389	Sharman English Medium School	13	1	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
390	Sharman English Medium School	13	1	8	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
391	Sharman English Medium School	14	1	9	5	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	0	1	1
392	Sharman English Medium School	14	1	9	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
393	Sharman English Medium School	14	1	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
394	Sharman English Medium School	14	1	9	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
395	Sharman English Medium School	15	2	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
396	Sharman English Medium School	15	2	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
397	Sharman English Medium School	15	1	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
398	KLE GA School	13	2	8	5	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
399	KLE GA School	13	2	8	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
400	KLE GA School	13	1	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
401	KLE GA School	14	1	8	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
402	KLE GA School	13	2	8	5	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1
403	KLE GA School	13	2	8	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
404	KLE GA School	13	1	8	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
405	KLE GA School	13	1	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
406	KLE GA School	13	2	8	5	1	0	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
407	KLE GA School	13	1	8	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
408	KLE GA School	13	1	8	5	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0	1	1
409	KLE GA School	13	1	8	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
410	KLE GA School	13	2	8	5	1	1	1	1	1	1	1	1	1	0	0	0	1	1	0	0	2	1
411	KLE GA School	13	1	8	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
412	KLE GA School	13	1	8	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
413	KLE GA School	13	1	8	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
414	KLE GA School	13	2	8	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
415	KLE GA School	13	1	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
416	KLE GA School	13	1	8	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
417	KLE GA School	13	1	8	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
418	KLE GA School	14	1	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
419	KLE GA School	14	2	9	6	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0	2	1
420	KLE GA School	14	1	9	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
421	KLE GA School	14	1	9	6	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
422	KLE GA School	14	1	9	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
423	KLE GA School	14	2	9	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
424	KLE GA School	14	1	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
425	KLE GA School	14	1	9	5	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
426	KLE GA School	14	1	9	6	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1
427	KLE GA School	14	1	9	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
428	KLE GA School	14	2	9	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
429	KLE GA School	14	2	9	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
430	KLE GA School	14	1	9	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
431	KLE GA School	14	1	9	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
432	KLE GA School	14	1	9	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
433	KLE GA School	14	2	9	5	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1
434	KLE GA School	14	2	9	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
435	KLE GA School	14	1	9	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
436	KLE GA School	14	1	9	4	1	2	88	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
437	KLE GA School	14	1	9	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
438	KLE GA School	15	2	10	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
439	KLE GA School	16	2	10	4	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
440	KLE GA School	15	1	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
441	KLE GA School	15	1	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
442	KLE GA School	16	2	10	6	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
443	KLE GA School	15	1	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
444	KLE GA School	15	1	10	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
445	KLE GA School	16	1	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
446	KLE GA School	16	2	10	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
447	KLE GA School	15	1	10	7	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
448	KLE GA School	15	1	10	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1

id	school	age	sex	std	famsize	housetype	msa	sba	gram	catalase	coag_slide	coag_tube	urease	P	E	CD	COT	CX	CIP	AMC	LZ	outcome	sa
449	KLE GA School	15	1	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
450	KLE GA School	15	2	10	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
451	KLE GA School	15	2	10	4	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
452	KLE GA School	15	1	10	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
453	KLE GA School	15	1	10	5	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1
454	Maratha Mandal English Medium School	6	1	1	5	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	1	1
455	Maratha Mandal English Medium School	6	2	1	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
456	Maratha Mandal English Medium School	6	2	1	5	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
457	Maratha Mandal English Medium School	6	2	1	5	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0	1	1
458	Maratha Mandal English Medium School	7	1	2	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
459	Maratha Mandal English Medium School	7	2	2	4	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1
460	Maratha Mandal English Medium School	7	1	2	4	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
461	Maratha Mandal English Medium School	8	1	3	4	1	1	1	1	1	1	1	1	1	0	0	0	0	1	0	0	1	1
462	Maratha Mandal English Medium School	8	1	3	5	1	2	2	3	99	99	99	99	99	99	99	99	99	99	99	99	3	0
463	Maratha Mandal English Medium School	8	1	3	5	1	0	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
464	Maratha Mandal English Medium School	9	2	3	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
465	Maratha Mandal English Medium School	9	2	4	5	1	2	2	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
466	Maratha Mandal English Medium School	9	2	4	5	1	1	1	1	1	1	1	1	1	0	0	1	0	1	0	0	1	1
467	Maratha Mandal English Medium School	9	1	4	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0
468	Maratha Mandal English Medium School	10	1	5	5	1	2	88	2	1	0	0	99	99	99	99	99	99	99	99	99	3	0

Annexure

Prevalence of Nasal carriage of Staphylococcus aureus among school children of an urban area

Keys used in Master Data Sheet

S No	Variable Name	Full Form	Details of Codes
1	id	Identification No of participants	
2	school	School Name	
3	age	Age of Student	
4	sex	Sex of Student	1 - Male, 2 - Female
5	std	Class of Student	actual class studying now in digits (eg 3 for student studying in class third)
6	famsize	Number of members in the family currently living together	Actual number of members
7	housetype	Type of House	Brick - 1, Mud-thatch - 2, Not known - 99
8	msa	Colony Morphology on Mannitol Salt Agar	Sensitive - 0, Resistant - 1, Intermediate
9	sba	Colony Morphology on Sheep Blood Agar	No colony - 0, beta-hemolysis with small circular white colony - 1, Beta-hemolysis with minute colony - 2, Others - 88
10	gram	Gram Stain Confirmation	Gram positive Cocci in cluster - 1, Gram positive micrococci - 2, Gram Positive Bacilli - 3
11	catalase	Result of Catalase Test	Positive - 1, Negative - 0, Not applicable - 99
12	coag_slide	Result of Slide Coagulase Test	Positive - 1, Negative - 0, Not applicable - 99
13	coag_tube	Result of Tube Coagulase Test	Positive - 1, Negative - 0, Not applicable - 99
14	urease	Result of Urease Test	Positive - 1, Negative - 0, Not applicable - 99
15	P	Antibiotic Sensitivity - Penicillin	Sensitive - 0, Resistant - 1, Not applicable - 99
16	E	Antibiotic Sensitivity - Erythromycin	Sensitive - 0, Resistant - 1, Not applicable - 99
17	CD	Antibiotic Sensitivity - Clindamycin	Sensitive - 0, Resistant - 1, Not applicable - 99
18	COT	Antibiotic Sensitivity - Cotrimoxazole	Sensitive - 0, Resistant - 1, Not applicable - 99
19	CX	Antibiotic Sensitivity - Cefoxitin	Sensitive - 0, Resistant - 1, Not applicable - 99
20	CIP	Antiobiotic Sensitivity - Ciprofloxacin	Sensitive - 0, Resistant - 1, Not applicable - 99
21	AMC	Antibiotic Sensitivity - Amoxyclav	Sensitive - 0, Resistant - 1, Not applicable - 99
22	LZ	Antibiotic Sensitivity - Linezolid	Sensitive - 0, Resistant - 1, Not applicable - 99
23	outcome	Outcome of nasal smear	MSSA - 1, MRSA - 2, Commensals - 3
24	sa	Staphylococcus aureus present in nasal smear	Yes - 1, No - 0