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RESEARCH

Multidrug Resistant Pathogenic *Staphylococcus aureus* in the Pimples

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ABSTRACT

Staphylococcus aureus is a common pathogen that colonizes and produces disease in a variety of hosts. Present study was conducted to identify the presence of pathogenic *Staphylococcus aureus* collected from pus samples of pimples from 18-25 yrs aged students from Medinipur-West, India and to characterize in respect of hydrolyzing property of protein, hemolytic activity, antibiotic susceptibility and biofilm forming ability. In the present study 35 bacterial isolates were identified as *Staphylococcus aureus*. All of them were found to hydrolyze protein and 94.28% of them were hemolytic. Furthermore, all *Staphylococcus aureus* isolates were found resistant to Ciprofloxacin, Tetracycline, Erythromycin, Trimethoprim-sulfamethoxazole, Clindamycin. On the other hand, 62.85% of them were resistant to Ampicillin, Penicillin, Gentamicin, Streptomycin, Chloramphenicol, Rifampicin. 25.71% were resistant to Methicillin and also possessed *mecA* as well as *lukS/F-PV*. But 77.78% of the methicillin-resistant *Staphylococcus aureus* isolates were found to be resistant to Vancomycin and 82.85% of *Staphylococcus aureus* isolates can produce moderate amount of biofilm. Hence, *Staphylococcus aureus* isolates who were MRSA, VRSA and also harbored *lukS/F-PV* may be appeared as the potential threat to the community health and also treatment plan in future.

Key words: *Staphylococcus aureus*, 16S rRNA, hemolysis, *mecA*, *lukS/F-PV*, biofilm.

List of abbreviation:

1. CA-MRSA:Community associated Methicillin resistant *Staphylococcus aureus*
2. MRSA: Methicillin resistant *Staphylococcus aureus*
3. PVL: Panton-Valentin leukocidin
4. h: hour

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5. sec: second
6. s: sensitive
7. r: resistant
8. Tet: Tetracycline
9. Ery: Erythromycin
10. Trimeth: Trimethoprim-sulfamethoxazole
11. Clin: Clindamycin; Amp: Ampicillin
12. Pen: Penicillin
13. Gent: Gentamicin
14. Strep: Streptomycin
15. Chl: Chloramphenicol
16. Rif: Rifampicin
17. Vanc: Vancomycin
18. Oxa: Oxacillin.
19. MSSA: Methicillin sensitive *Staphylococcus aureus*

1. INTRODUCTION

Staphylococcus aureus is ubiquitous commensal bacterium on human skins and anterior nares. The organism has been reported to be a major human pathogen causing skin and tissue infections, pneumonia, septicemia and device associated infections (Lowy, 1998; Cosgrove et al., 2003; Deleo et al., 2010; Chambers et al., 2009). Pathogenicity of *Staphylococcus aureus* depends upon the production of several virulence factors such as coagulase, Staphylococcal nuclease, proteases, lipases and elastases (Palma et al., 1999; Sandel and McKillip, 2004; Prozan and novice, 1997).

Due to adaptation to different antibiotic the organism becomes multidrug resistant leaving few therapeutic options for the treatment against it. The emergence of methicillin (oxacillin) resistant *Staphylococcus aureus* (MRSA) creates a serious health problem globally (Deleo et al., 2010; Chambers et al., 2009; Hafeez et al., 2004; Saha et al., 2008). Boucher and Corey (2008) reported that the frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) infections increases continuously in hospital-associated settings and more recently, in community settings. In India methicillin resistant *Staphylococcus aureus* (MRSA) is now endemic and incidence of community acquired MRSA (CA-MRSA) have been found to increase (Patel et al., 2010; D'Souza et al., 2010). Several reports suggested that methicillin resistance is mediated by PBP-2a, a penicillin binding protein encoded by the *mecA* gene that permits the organism to grow and divide in the presence of methicillin and other beta-lactam antibiotics. Additionally, the *mecA* gene has been found to be located on a mobile genetic element called staphylococcal chromosome cassette (SCCmec) which is more easily transferred to other strains of *Staphylococcus aureus* (Inglis et al., 1988; Zhang et al., 2005). Besides, it was also reported that newly emerging community-associated MRSA (CA-MRSA) strains not only carry SCCmec but also possess Panton-Valentine leukocidin (PVL) virulence genes (Said-Salim et al., 2005; Vandenesch et al., 2003; Zhang et al., 2005). Panton-Valentine leukocidin is a bicomponent leukocidin encoded by two co-transcribed genes, *lukS-PV* and *lukF-PV* (*lukS/F-PV*) residing on a prophage and found to cause leukocyte destruction and tissue necrosis (Finck-Barbancon et al., 1993; Supersac et al., 1993).

Moreover, emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) has also been reported to be increased globally resulting in increased morbidity and mortality (Thati et al., 2011; De et al., 2006). Several reports have shown the emergence of low level and intermediate vancomycin-resistant *Staphylococcus aureus* (VRSA) in north India (Assadullah et al., 2003; Tiwari and Sen, 2006).

On the other hand, the organism has the ability to colonize healthy individuals asymptomatically and may spread among healthy individuals from *Staphylococcus aureus* carriers (Lowy, 1998). Additionally, Kluytmans et al. (1997) reported that the human nares are the primary niche for the colonization of *Staphylococcus aureus*. For colonization to the host body *Staphylococcus aureus* can produce biofilm through initially adhering to host epithelial surfaces and subsequently releasing some of their DNA as extracellular matrix to replicate as biofilm communities (Sadykov and Bayles, 2012; Archer et al., 2011; Iwase et al., 2010). CA-MRSA usually has been found to cause skin infections such as pimples and boils (Francis et al., 2005). Pimple (collection of pustules) is one of the forms of acne and a common problem among adolescents and young adults (Hanisah et al., 2009; Shen et al., 2012; Goulden et al., 1999). It is characterized by visible collection of pus within or beneath the epidermis (Akimoto et al., 1996; Thappa et al., 2009). Suggestion is there that prolonged hospital stays, indiscriminate use of antibiotics, lack of awareness, and receipt of antibiotics before coming to the hospital are some of the possible predisposing factors of MRSA emergence (Hafeez et al., 2004). Hence, in selection of appropriate treatment against these infections, knowledge of MRSA prevalence and the current antimicrobial profile are very crucial.

In this context, goal of the present study was to identify the presence of pathogenic *Staphylococcus aureus* from pus of pimples of young adults and characterize them in respect of several virulence properties, such as protein hydrolysis, hemolytic activity, antibiotic susceptibility and biofilm forming ability.

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2. MATERIALS AND METHODS

2.1. Collection of samples

Pus of pimples was collected from the students of both sexes of age group of 18-25 years from Medinipur-West, India during the period of January 2013 to August 2013. This study was conducted as per the human ethical guidelines and pus samples were collected with informed consent from the subjects and institutional authority.

2.2. Bacterial strains and culture method

Pus samples were collected aseptically, transferred into LB agar media and incubated overnight at 37ºC. The bacterial colonies obtained from the pus samples were cultured in sheep blood agar and incubated overnight at 37ºC under both aerobic and anaerobic conditions for 2 to 7 days.

2.3. Identification including biochemical characterization

The *Staphylococcus aureus* bacterial isolates obtained from pus samples were confirmed by PCR through detection of 16S rRNA (*Staphylococcus aureus* specific) (Monday and Bohac, 1999). Simultaneously, these isolates were identified as *Staphylococcus aureus* according to routine microbiological diagnostics, including Gram stain, colony morphology, catalase, coagulase, heat-stable nuclease production on thermonuclease test agar, mannitol salt agar fermentation (Ronald et al., 1995; Cruickshank et al., 1975; Quinn et al., 2002; Lachica et al., 1971). They were also characterized for protease activity on milk agar medium and hemolytic activity on sheep blood agar (15 ml of 5% sheep blood in Trypticase soy agar overlaid on 10 ml of blood agar base) according to Rodger et al. (1999). *Staphylococcus aureus* ATCC 25923 was used as the positive control.

2.4. Antibiotic susceptibility

Antibiotic susceptibility test was performed against the *Staphylococcus aureus* isolates by disc diffusion method. *Staphylococcus aureus* isolates were cultured on Muller Hinton agar and antibiotic discs were used for Ampicillin (50 µg), Penicillin (10 U), Gentamicin (10 µg), Streptomycin (100 µg), Trimethoprim-sulfamethoxazole (30 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Erythromycin (15 µg), Clindamycin (2 µg), Rifampicin (5 µg) and Vancomycin (30 µg) (HiMedia Laboratories Pvt. Ltd., India) according to standard recommendation (Bauer et al., 1966). Isolates were categorized as susceptible and resistant according to interpretative criteria developed by the Clinical and Laboratory Standards Institute (CLSI) (2005). *Staphylococcus aureus* ATCC 25923 was used as the quality control strain for disc diffusion.

Further, for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) resistance towards methicillin was determined followed by the method mentioned by the CLSI, using Muller Hinton agar plate supplemented with NaCl (0.68 mol/L) and Oxacillin (6 mg/ml, w/v) (HiMedia Laboratories Pvt. Ltd., India) (Coelho et al., 2007).

2.5. DNA extraction for PCR analysis

DNA from *Staphylococcus aureus* was extracted from overnight cultures in 10 ml of brain heart infusion broth with slight modifications of the method mentioned by Sambrook and Russel, (2001).

2.6. PCR analysis

Polymerase chain reaction was conducted for detection of 16S rRNA (*Staphylococcus aureus* specific), *mecA* genes (for detection of methicillin resistant strains) and *lukS/F-PV* (encoding Panton-Valentin leukocidin S/F proteins, determinant of CA-MRSA strain) using specific primer pair for each gene (Table 1). PCR amplification reactions were carried out by standard protocol in a thermal cycler (Eppendorf) (Monday and Bohac, 1999; Zhang et al., 2005; Lina et al., 1999).

2.7. Assay for biofilm formation

Biofilm formation on abiotic surfaces was estimated following the method described by Heilmann et al. (1996). *Staphylcoccus aureus* was allowed to grow overnight in tryptone soy broth (TSB) (HiMedia Laboratories Pvt. Ltd., India) supplemented with 0.25% glucose (TSB-glucose). Then culture was diluted 1:40 in TSB-glucose, and 200 µl of this cell suspension was inoculated to each well of 96-well polystyrene microtiter plates (sterile) (Tarson, India). After 18 *h* of incubation at 37°C, the wells were gently washed for three times with 200 µl of sterile phosphate-buffered saline (PBS). They were allowed to dry in air in an inverted position, and stained with 0.1% safranin for 30*s*. Next, wells were rinsed again, and the absorbance was determined at 490 *nm* using spectrophotometer (Schimadzu, Japan). Each assay was performed in triplicate in three separate experiments.

3. RESULTS AND DISCUSSION

3.1. Identification and characterization

Staphylococcus aureus is an important and frequent cause of wound infection and is responsible for a range of hospital and community-acquired infections (Kluytmans et al., 1997; Lowy, 1998; Patel et al., 2010). In the present study total 61 bacteria were isolated from pus samples collected from the subjects chosen and thirty five isolates (57.37%) were identified as *Staphylococcus aureus* those were Gram positive, smooth golden yellow colonies and also positive for catalase, coagulase, heat-stable nuclease production, fermentation of mannitol.

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After primary identification procedure, all these 35 isolates were further confirmed as *Staphylococcus aureus* by a PCR amplification of 16S rRNA (*Staphylococcus aureus* specific) (Monday and Bohac, 1999) (Figure 1). On the other hand all the *Staphylococcus aureus* isolates were found to have protease activity on casein agar plate (Table 2). It was also previously reported that during infection, near all strains of *Staphylococcus aureus* produces proteases that enables it to invade and destroy host tissues and metastasize to other sites (Foster and Hook, 1998; Prozan and novice, 1997). Present study showed that 33 (94.28%) of *Staphylococcus aureus* isolates were able to produce clearing zone surrounding their growth on blood agar media (Table 2) demonstrating that they can produce hemolysin. Several reports also suggested that *Staphylococcus aureus* produces hemolysins for contribution to its pathogenicity (Menestrina et al., 2001).

3.2. Antibiotic susceptibility and genetic marker of CA-MRSA

On other hand, *Staphylococcus aureus* has been found to become resistant to most classes of antimicrobial agents and creates a serious treatment problem (Saha et al., 2008). In the present study we found that all of *Staphylococcus aureus* isolates were resistant to Ciprofloxacin, Tetracycline, Erythromycin, Clindamycin, Trimethoprim-sulfamethoxazole those are usually used for the treatment of pimples (acne) (Leyden, 1997 and 2001; Margolis, 2006). Moreover, 22 (62.86%) of these *Staphylococcus aureus* isolates were also found to be resistant to Ampicillin, Penicillin, Gentamicin, Streptomycin, Chloramphenicol, Rifampicin (Table 2). Besides, 9 (25.71%) of them were resistant to Methicillin on agar plate containing Oxacillin (Table 2). Present study also showed that all phenotypically identified (Table 2) methicillin-resistant *Staphylococcus aureus* isolates possessed *mecA* (Figure 2). These observations indicate that presence of *mecA* gene among phenotypically identified methicillin-resistant *Staphylococcus aureus* isolates (Figure 2) may be responsible for conferring resistant to Methicillin and also other beta-lactam antibiotics (Inglis et al., 1988). Moreover, it has been also shown that clinically, community-associated MRSA infection often are present as pyogenic skin and soft-tissue infections in healthy individuals (King et al., 2006). Beside this, it was reported earlier that CA-MRSA strains are usually resistant to β-lactams but susceptible to other antimicrobials like Trimethoprim-sulfamethoxazole, Clindamycin and Tetracyclines (Deresinski, 2005), but our study strains did not follow this (Table 2) which may be due to different geographic location (Gupta et al., 2009).

Additionally, we found that 7 (77.78%) of the methicillin-resistant *Staphylococcus aureus* isolates were also resistant to Vancomycin (Table 2), the glycopeptide antibiotic, which was thought to be effective for the treatment of many of multidrug resistant MRSA isolates (Rajaduraipandi, 2006). However, Rajaduraipandi (2006) also reported that resistance even to Vancomycin has been found to be emerged at present. Thati et al. (2011) has shown the presence of VRSA strains in India.

Present study also revealed that 10 (28.57%) *Staphylococcus aureus* isolates harbored *lukS/F-PV* which was reported to be a stable genetic marker of CA-MRSA strains and to be associated with primary skin infections and occasionally necrotizing pneumonia (Lina et al., 1999; Naimi et al., 2001; Gillet et al., 2002). Interestingly, 9 of them were found to possess both *lukS/F-PV* and *mecA* genes (Table 3). Moreover, Vandenesch et al., (2003) had also shown the presence of PVL locus in CA-MRSA strains. Suggestion was there that the combination of the Panton-Valentine leukocidin determinant with the *mecA* gene have been appeared to be involved for the creation of a superadapted *Staphylococcus aureus* strain that is spreading in the community (Dufour et al., 2002). Hence, 7 (20%) of *Staphylococcus aureus* isolates were MRSA, VRSA and also possessed *lukS/F-PV* which demonstrate that these isolates may be appeared as the potential threat to the public health in future and also may be responsible for spread of severe soft tissue infection.

3.3. Biofilm formation ability

On the other hand, several studies have shown that like many other bacterial organism for the colonization *Staphylococcus aureus* can produce biofilm (slime) on human nares, prosthetic surfaces, enabling it to persist by evading host defenses and antimicrobials (Sadykov and Bayles, 2012; Archer et al., 2011). Present study also revealed that 29 (82.85%) of the *Staphylococcus aureus* isolates were able to form moderate biofilm (Figure 3). Hence, it can be interpreted that biofilm production by the present isolates may be responsible for acting as a protective barrier to antibiotics (Bellew et al., 2011; Coenye et al., 2007) and in that way to be helpful for the progression of the infection.

Results of the present study indicate that the study subjects with pimple may act as a reservoir for community acquired-MRSA, sometimes VRSA, and also multidrug resistant *Staphylococcus aureus* strains (other than MRSA and VRSA) (Levy et al., 2005; Fanelli et al., 2011) which is highly significant in terms of spread of infection in the community and treatment plan.

SUMMARY POINTS

1. All the results of the present study revealed that the *Staphylococcus aureus* isolates (57.37%) having pathogenicity were present in the pus samples collected from pimples.
2. They were all resistant to Ciprofloxacin, Tetracycline, Erythromycin, Trimethoprim-sulfamethoxazole, Clindamycin. Moreover, 62.85% of them were resistant to Ampicillin, Penicillin, Gentamicin, Streptomycin, Chloramphenicol, Rifampicin used in the present study.
3. *lukS/F-PV* harboring Methicillin-resistant *Staphylococcus aureus* and Vacnomycin-resistant *Staphylococcus aureus* were also present in the pus samples collected from pimples.
4. All the *Staphylococcus aureus* isolates were able to produce biofilm which demonstrates that pimples may act as a reservoir of multidrug resistant *Staphylococcus aureus* strains.

FUTURE ISSUES

1.Is there any difference in SCC*mec* gene between CA-MRSA in the present study and hospital associated MRSA?

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2.What is the reason for developing resistance towards Vancomycin among the VRSA in this study?

DISCLOSURE STATEMENT

This article is original, has not been submitted for publication in other journals and has not yet been published either wholly or in part. This study carried out on human beings, was taken after the prior approval of institutional ethical committee. This study was conducted as per the human ethical guidelines and pus samples were collected with informed consent from the subjects and institutional authority. Subjects were selected from the community.

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REFERENCES

1. Akimoto Y, Nishimura H, Omata H, Shibutani J, Kaneko K et al. Cefaclor concentration in pus from abscess caused byodontogenic infection after a single oral administration. *Gen Pharmacol* , 1996, 27, 177-179.
2. Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME. *Staphylococcus aureus* biofilms: properties, regulation, and roles in human disease. *Virulence,* 2011, 2, 445-459.
3. Assadullah S, Kakru DK, Thoker MA, Bhat FA, Hussain N, Shah A. Emergence of low level vancomycin resistance in MRSA. *Indian J Med Microbiol,* 2003, 21, 196-198.
4. Bauer AW, Kirby WMM, Sheris JC, Turck M. Antibiotics susceptibility testing by standardized single disk method. *Am J Clin Pathol* , 1966, 45, 493-496.
5. Bellew S, Thiboutot D, Del Rosso JQ. Pathogenesis of acne vulgaris: what's new, what's interesting and what may be clinically relevant. *J Drugs Dermatol* , 2011, 10, 582-585.
6. Boucher HW, Corey GR. Epidemiology of Methicillin-Resistant *Staphylococcus aureus*. *Clin Infect Dis,* 2008, 46(Supplement 5), S344-S349.
7. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol,* 2009, 7(9), 629-641.
8. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standards. CLSI, Wayne, PA, 2005.
9. Coelho SMO, Menezes RA, Soares LC, Pereira IA, Gomes LP, Souza MMS. Mapeamento do perfil de resistência e detecção do gene *mecA* em *Staphylococcus aureus* e *Staphylococcus intermedius* oxacilina-resistentes isolados de espécies humanas e animais. *Cienc Rura* , 2007, 37(1), 195-200.
10. Coenye T, Peeters E, Nelis HJ. Biofilm formation by *Propionibacterium acnes* is associated with increased resistance to antimicrobial agents and increased production of putative virulence factors. *Res Microbiol* , 2007, 158, 386-392.
11. Cosgrove SE, Sakoulas G, Perencevich EN, Schwaber MJ, Karchmer AW, Carmeli Y. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* bacteremia: a meta-analysis. *Clin Infect Dis* , 2003, 36, 53-59.
12. Cruickshank R, Duguid JP, Marmion BP, Swain RHA. Medical Microbiology. Vol. 2, 12th ed, 1975, Chruchill Livingstone, New York.
13. De Lassence A, Hidri N, Timsit JF, Joly-Guillou ML, Thiery G et al. Control and outcome of a large outbreak of colonization and infection with glycopeptide-intermediate *Staphylococcus aureus* in an intensive care unit. *Clin Infect Dis* , 2006, 42, 170-178.
14. Deleo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated methicillin-resistant *Staphylococcus aureus*. *Lancet,* 2010, 375, 1557-1568.
15. Deresinski S. Methicillin-resistant *Staphylococcus aureus*: An evolutionary, epidemiologic, and therapeutic odyssey. *Clin Infect Dis* , 2005, 40, 562-573.
16. D'Souza N, Rodrigues C, Mehta A. Molecular characterization of methicillin-resistant *Staphylococcus aureus* with emergence of epidemic clones of sequence type (ST) 22 and ST 772 in Mumbai, India. *J Clin Microbiol* , 2010, 48, 1806-1811.
17. Dufour P, Gillet Y, Bes M, Lina G, Vandenesch F et al. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in France: Emergence of a Single Clone That Produces Panton-Valentine Leukocidin. *Clin Infect Dis,* 2002, 35, 819-824.
18. Fanelli M, Kupperman E, Lautenbach E, Edelstein PH, Margolis DJ. Antibiotics, acne, and *Staphylococcus aureus* colonization. *Arch Dermatol,* 2011, 147(8), 917-921.
19. Finck-Barbancon V, Duportail G, Meunier O, Colin DA. Pore formation by a two-component leukocidin from *Staphylococcus aureus* within the membrane of human polymorphonuclear leukocytes. *Biochim Biophys Acta,* 1993, 1182, 275-282.
20. Foster TJ, Hook M. Surface protein adhesins of *Staphylococcus aureus*. *Trends Microbiol,* 1998, 6(12), 484-488.
21. Francis JS, Doherty MC, Lopatin U, Johnston CP, Sinha G et al. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant *Staphylococcus aureus* carrying the Panton-Valentine leukocidin genes. *Clin Infect Dis* , 2005, 40, 100-107.
22. Gillet Y, Issartel B, Vanhems P, Fournet JC, Lina G, Bes M. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet,* 2002, 359, 753-759.
23. Goulden V, Stables GI, Cunliffe WJ. Prevalence of facial acne in adults. *J Am Acad Dermatol,* 1999, 41(4), 577-580.
24. Gupta V, Datta P, Rani H, Chander J. Inducible clindamycin resistance in *Staphylococcus aureus*: A study from North India. *J Postgrad Med,* 2009, 55, 176-179.
25. Hafeez R, Chughtai A, Aslam M. Prevalence and antimicrobial susceptibility of MRSA. *Int J Path,* 2004, 2(1), 10-15.
26. Hanisah A, Omar K, Shah SA. Prevalence of acne and its impact on the quality of life in school-aged adolescents in Malaysia. *J Prim Health Care,* 2009, 1(1), 20-25.
27. Heilmann C, Gerke C, Perdreau-Remington F, Gotz F. Characterization of Tn*917* insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. *Infect Immun,* 1996, 64, 277-282.
28. Inglis B, Matthews PR, Stewart PR. The expression in *Staphylococcus aureus* of cloned DNA encoding methicillin resistance. *J Gen Microbiol* , 1988, 134, 1465.
29. Iwase T, Uehara Y, Shinji H, Tajima A, Seo H et al. *Staphylococcus epidermidis* Esp inhibits *Staphylococcus aureus* biofilm formation and nasal colonization. *Nat,* 2010, 465, 346-349.

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30. King MD, Humphrey BJ, Wang YF, Kourbatova EV, Ray SM, Blumberg HM. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft tissue infections. *Ann Intern Med,* 2006, 144, 309-317.
31. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev*, 1997, 10(3), 505-520.
32. Lachica RVF, Genigeorgis C, Hoeprich PD. Metachromatic agar-diffusion methods for detecting staphylococcal nuclease activity. *Appl Microbiol*, 1971, 21, 585-587.
33. Levy RM, Leyden JJ, Margolis DJ. Colonization rates of *Streptococcus pyogenes* and *Staphylococcus aureus* in the oropharynx of a young adult population. *Clin Microbiol Infect,* 2005, 11(2), 153-155.
34. Leyden JJ. Therapy for acne vulgaris. *N Engl J Med,* 1997, 336(16), 1156-1162.
35. Leyden JJ. Current issues in antimicrobial therapy for the treatment of acne. *J Eur Acad Dermatol Venereol,* 2001, 15, 51-55.
36. Lina G, Piemont Y, Godail-Gamot F, Bes M, Peter MO, Gauduchon V. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis,* 1999, 29, 1128-1132.
37. Lowy FD. *Staphylococcus aureus* infections. *New Engl J Med,* 1998, 339, 520-532.
38. Margolis DJ. Antibiotics, acne, and upper respiratory tract infections. *LDI Issue. Brief,* 2006, 11(4), 1-4.
39. Menestrina G, Serra MD, Prevost G. Mode of action of beta-barrel pore-forming toxins of the staphylococcal alpha-hemolysin family. *Toxicon,* 2001, 39, 1661-1672.
40. Monday SR, Bohach GA. Use of multiplex PCR to detect classical and newly described pyrogenic toxin genes in Staphylococcal Isolates. *J Clin Microbial,* 1999, 37, 3411-3414.
41. Naimi TS, LeDell KH, Boxrud DJ, Groom AV, Steward CD, Johnson SK. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996–1998. *Clin Infect Dis* , 2001, 33, 990-996.
42. Palma M, Haggar A, Flock J. Adherence of *Staphylococcus aureus* is enhanced by an endogenous secreted protein with broad binding activity. *J Bacteriol,* 1999, 181(9), 2840-2845.
43. Patel AK, Patel KK, Patel KR, Shah S, Dileep P. Time trends in the epidemiology of microbial infections at a tertiary care center in west India over last 5 years. *J Assoc Physicians India,* 2010, 58, 37-40.
44. Projan SJ, Novick RP. The molecular basis of pathogenicity. *In the Staphylococci in human disease* (ED. By Crossley KB, Archer GL), 1997, pp. 55-81. New York: Churchill Livingstone.
45. Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonard FC, Maguire D. Veterinary microbiology and microbial disease. Blackwell Science Ltd, 2002.
46. Rajaduraipandi K, Mani KP, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. *Indian J Med Microbiol* , 2006, 24, 34-38.
47. Rodgers JD, Cullagh JJ, McNamee PT, Smyth JA, Hywel J. Comparison of *S. aureus* recovered from personnel in a poultry hatchery and in broiler parent farms with those isolated from skeletal disease in broilers. *Vet Microbiol* , 1999, 69, 189-198.
48. Ronald MA, Brown AE, Parks LC. In *Laboratory Manual-Experimental Microbiology* (ED. By James M Smith), 1995, pp. 68-93. USA.
49. Sadykov MR, Bayles KW. The control of death and lysis in staphylococcal biofilms: a coordination of physiological signals. *Curr Opin Microbiol* , 2012, 15, 211-215.
50. Saha B, Singh AK, Ghosh A, Bal M. Identification and characterization of a vancomycin resistant *Staphylococcus aureus* isolated from Kolkata (South Asia). *J Med Microbiol* , 2008, 57, 72-79.
51. Said-Salim B, Mathema B, Braughton K, Davis S, Sinsimer D et al. Differential distribution and expression of Panton-Valentine leucocidin among community-acquired methicillin-resistant *Staphylococcus aureus* strains. *J Clin Microbiol* , 2005, 43, 3373-3379.
52. Sambrook J, Russel DW. Molecular Cloning. In *a Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
53. Sandel MK, McKillip JL. Virulence and recovery of Staphylococcus aureus relevant to the food industry using improvements on traditional approaches. *Food Control,* 2004, 15(1), 5-10.
54. Shen Y, Wang T, Zhou C, Wang X, Ding X et al. Prevalence of acne vulgaris in chinese adolescents and adults: a community-based study of 17,345 subjects in six cities. *Acta Derm Venereol,* 2012, 92(1), 40-44.
55. Supersac G, Prevost G, Piemont Y. Sequencing of leucocidin R from *Staphylococcus aureus* P83 suggests that staphylococcal leucocidins and gamma-hemolysin are members of a single, two-component family of toxins. *Infect Immun,* 1993, 61, 580-587.
56. Thappa D, Balaji A, Kumari R. Scoring systems in acne vulgaris. *Indian J Dermatol Venereol Leprol,* 2009, 75(3), 323-336.
57. Thati V, Shivannavar CT, Gaddad SM. Vancomycin resistance among methicillin resistant *Staphylococcus aureus* isolates from intensive care units of tertiary care hospitals in Hyderabad. *Indian J Med Research,* 2011, 134, 704-708**.**
58. Tiwari HK, Sen MR. Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. *Infect. Dis,* 2006, 6, 156.
59. Vandenesch F, Naimi T, Enright MC, Lina G, Nimmo GR et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine Leukocidin genes: Worldwide Emergence. *Emerg Infect Dis,* 2003, 9(8), 978-984.
60. Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbial,* 2005, 43(10), 5026-5033.

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Table 1

Primers for screening of 16S r RNA, *mecA* and *lukS/F-PV* gene

Primer	Sequences (5´- 3´)	Amplicon size (bp)
16S r RNA p1	GTA GGT GGC AAG CGT TAT CC	228
16S r RNA p2	CGCACATCAGCGTCAG	
mecA p1	GTG AAG ATA TAC CAA GTG ATT	147
mecA p2	ATG CGC TAT AGA TTG AAA GGA T	
Luk-PV-1	ATCATTAGGTAAAATGTCTGGACATGATCCA	433
Luk-PV-2	GCATCAAGTGTATTGGATAGCAAAAGC	

Table 2

Biochemical characterization of *Staphylococcus aureus* isolates

Samples	Protease activity	Hemolytic activity	Antibiotic susceptibility
SBS1	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^s$, Oxa^s
SBS2	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^s, Pen^s, $Gent^s$, $Strep^s$, Chl^s, Rif^s, $Vanc^s$, Oxa^s
SBS3	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^s, Pen^s, $Gent^s$, $Strep^s$, Chl^s, Rif^s, $Vanc^s$, Oxa^s
SBS4	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^s$, Oxa^s
SBS5	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^s$, Oxa^s
SBS6	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^s$, Oxa^r
SBS7	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^s, Pen^s, $Gent^s$, $Strep^s$, Chl^s, Rif^s, $Vanc^s$, Oxa^s
SBS8	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^s$, Oxa^r
SBS9	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^r$, Oxa^r
SBS10	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^s, Pen^s, $Gent^s$, $Strep^s$, Chl^s, Rif^s, $Vanc^s$, Oxa^s
SBS11	+	-	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^s$, Oxa^s
SBS12	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^r$, Oxa^r
SBS13	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^s, Pen^s, $Gent^s$, $Strep^s$, Chl^s, Rif^s, $Vanc^s$, Oxa^s
SBS14	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^r$, Oxa^r
SBS15	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^s$, Oxa^s
SBS16	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^r$, Oxa^r
SBS17	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^s$, Oxa^s
SBS18	+	+	Tet^r, Ery^r, $Trimeth^r$, $Clin^r$, Amp^r, Pen^r, $Gent^r$, $Strep^r$, Chl^r, Rif^r, $Vanc^s$, Oxa^s

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SBS19	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^r, Pen^r, Gent^r, Strep^r, Chl^r, Rif^r, Vanc^s, Oxa^s
SBS20	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^s, Pen^s, Gent^s, Strep^s, Chl^s, Rif^s, Vanc^s, Oxa^s
SBS21	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^r, Pen^r, Gent^r, Strep^r, Chl^r, Rif^r, Vanc^s, Oxa^s
SBS22	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^r, Pen^r, Gent^r, Strep^r, Chl^r, Rif^r, Vanc^r, Oxa^r
SBS23	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^s, Pen^s, Gent^s, Strep^s, Chl^s, Rif^s, Vanc^s, Oxa^s
SBS24	+	+	Tet^s, Ery^s, Trimeth^s, Clin^s, Amp^s, Pen^s, Gent^s, Strep^s, Chl^s, Rif^s, Vanc^s, Oxa^s
SBS25	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^r, Pen^r, Gent^r, Strep^r, Chl^r, Rif^r, Vanc^s, Oxa^s
SBS26	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^s, Pen^s, Gent^s, Strep^s, Chl^s, Rif^s, Vanc^s, Oxa^s
SBS27	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^r, Pen^r, Gent^r, Strep^r, Chl^r, Rif^r, Vanc^r, Oxa^r
SBS28	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^s, Pen^s, Gent^s, Strep^s, Chl^s, Rif^s, Vanc^s, Oxa^s
SBS29	+	-	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^r, Pen^r, Gent^r, Strep^r, Chl^r, Rif^r, Vanc^s, Oxa^s
SBS30	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^s, Pen^s, Gent^s, Strep^s, Chl^s, Rif^s, Vanc^s, Oxa^s
SBS31	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^s, Pen^s, Gent^s, Strep^s, Chl^s, Rif^s, Vanc^s, Oxa^s
SBS32	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^s, Pen^s, Gent^s, Strep^s, Chl^s, Rif^s, Vanc^s, Oxa^s
SBS33	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^r, Pen^r, Gent^r, Strep^r, Chl^r, Rif^r, Vanc^s, Oxa^s
SBS34	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^s, Pen^s, Gent^s, Strep^s, Chl^s, Rif^s, Vanc^s, Oxa^s
SBS35	+	+	Tet^r, Ery^r, Trimeth^r, Clin^r, Amp^r, Pen^r, Gent^r, Strep^r, Chl^r, Rif^r, Vanc^r, Oxa^r

N.B. s: sensitive; r: resistant; Tet: Tetracycline; Ery: Erythromycin; Trimeth: Trimethoprim-sulfamethoxazole; Clin: Clindamycin; Amp: Ampicillin; Pen^r: Penicillin; Gent: Gentamicin; Strep: Streptomycin; Chl: Chloramphenicol: Rif: Rifampicin; Vanc: Vancomycin; Oxa: Oxacillin.

Table 3

Genotypic characterization of *Staphylococcus aureus* isolates and detection of CA-MRSA strains

Samples	Strain characteristics[a]	Genotype[c] 16S rRNA	*mecA*	*lukS/F-PV*
SBS1	PVL(-)MSSA	+	-	-
SBS2	PVL(-)MSSA	+	-	-
SBS3	PVL(+)MSSA	+	-	+
SBS4	PVL(-)MSSA	+	-	-
SBS5	PVL(-)MSSA	+	-	-
SBS6	PVL(+)MRSA	+	+	+
SBS7	PVL(-)MSSA	+	-	-
SBS8	PVL(+)MRSA	+	+	+
SBS9	PVL(+)MRSA	+	+	+

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SBS10	PVL(-)MSSA	+	-	-
SBS11	PVL(-)MSSA	+	-	-
SBS12	PVL(+)MRSA	+	+	+
SBS13	PVL(-)MSSA	+	-	-
SBS14	PVL(+)MRSA	+	+	+
SBS15	PVL(-)MSSA	+	-	-
SBS16	PVL(+)MRSA	+	+	+
SBS17	PVL(-)MSSA	+	-	-
SBS18	PVL(-)MSSA	+	-	-
SBS19	PVL(-)MSSA	+	-	-
SBS20	PVL(-)MSSA	+	-	-
SBS21	PVL(-)MSSA	+	-	-
SBS22	PVL(+)MRSA	+	+	+
SBS23	PVL(-)MSSA	+	-	-
SBS24	PVL(-)MSSA	+	-	-
SBS25	PVL(-)MSSA	+	-	-
SBS26	PVL(-)MSSA	+	-	-
SBS27	PVL(+)MRSA	+	+	+
SBS28	PVL(-)MSSA	+	-	-
SBS29	PVL(-)MSSA	+	-	-
SBS30	PVL(-)MSSA	+	-	-
SBS31	PVL(-)MSSA	+	-	-
SBS32	PVL(-)MSSA	+	-	-
SBS33	PVL(-)MSSA	+	-	-
SBS34	PVL(-)MSSA	+	-	-
SBS35	PVL(+)MRSA	+	+	+

[a] MRSA: Methicillin resistant *Staphylococcus aureus*; MSSA: Methicillin sensitive *Staphylococcus aureus*; PVL: Panton-Valentin leukocidin.
[b]16S rRNA, *Staphylococcus* genus specific; *lukS/F-PV* genes encode Panton-Valentin leukocidin S/F proteins; *mecA*, determinant of methicillin resistance; + denotes positive for gene; – denotes negative for gene.

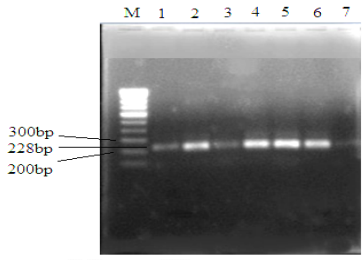

Figure 1
Agarose gel electrophoresis of PCR amplified products on 1% agarose gel for detection of 16S rRNA (*Staphylococcus aureus* specific) to confirm the *Staphylococcus aureus* isolates. Lane M: 100 bp DNA ladder; Lane 1: PCR amplified 16S rRNA of SBS1; Lane 2: PCR amplified 16S rRNA of SBS2; Lane 3: PCR amplified 16S rRNA of SBS3; Lane 4: PCR amplified 16S rRNA of SBS4; Lane 5: PCR amplified 16S rRNA of SBS6; Lane 6: positive control for 16S rRNA.

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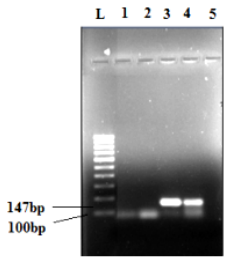

Figure 2

Agarose gel electrophoresis of PCR amplified products on 1% agarose gel for detection of methicillin resistance (*mecA*) among the *Staphylococcus aureus* isolates. *mecA* genes of phenotypically identified methicillin resistant *Staphylococcus aureus* isolates were amplied by PCR and electrophoresed on 1% agarose gel. (A) Lane L: 100bp DNA ladder; Lane 1: *mecA* negative; Lane 2: *mecA* negative; Lane 3: PCR fragment of *mecA*; Lane 4: PCR fragment of *mecA* of positive control.

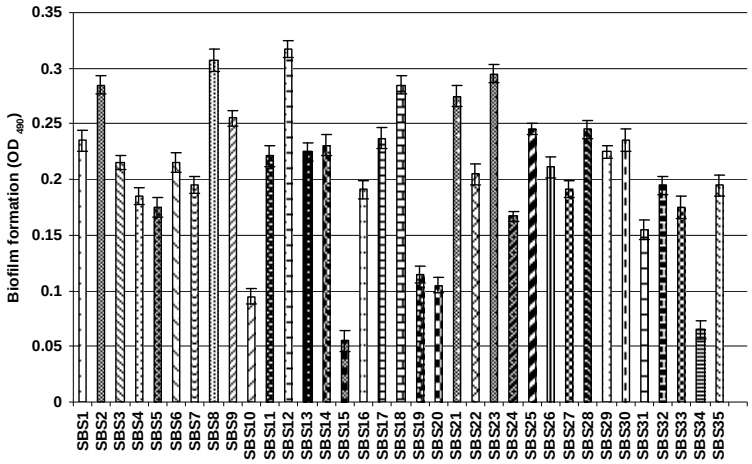

Figure 3

Biofilm formation by *Staphylococcus aureus* isolates. Biofilm formation at 37 °C for 18 *h* in *Staphylococcus aureus* isolates are presented. OD $_{490}$ values are representative of quantity of biofilm produced. Values are expressed as Mean ± SD.

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