Original Research Article

Study of the virulence factors and antimicrobial susceptibility profile of staphylococcus aureus isolated from clinical samples at tertiary care centre

Vedavati B. I.[1], Halesh L.H.[2], Koppad M.[3], Premalatha D. E.[4], Akshatha. Y. J.[5]

[1]Dr.Vedavati B. I., Tutor, [2]Dr. Halesh L.H., Professor and HOD, [3]Dr. Mallikarjun Koppad, Associate Professor, [4]Dr. D. E. Premalatha, Assistant Professor, [5]Dr. Akshatha Y.J., Tutor, all authors are affiliated with Department of Microbiology, Shimoga Institute of Medical Sciences, Shivamogga, Karnataka, India.

Corresponding Author: Dr. Akshatha Y. J., Tutor, Department of Microbiology, SIMS, Shivamogga, Karnataka, India. E-mail- yjakshatha9@gmail.com

Abstract

Introduction: Staphylococcus aureusis an important cause of healthcare-associated infections. The increasing resistance of this pathogen to various antibiotics complicates treatment. Present study was conducted to isolate staphylococcus aureus from various clinical samples, to study the few virulence factors, antibiogram and to detect methicillin resistance among them. **Materials and Methods:** Present study was done over a period of six months. The study was conducted on 100 staphylococcus aureus isolated from various clinical samples. The organisms were identified as per standard conventional methods. The antibiotic sensitivity testing of the isolates was done by Kirby Bauer's Disk Diffusion method according to CLSI guidelines. These strains were screened for DNase production, beta hemolytic property and slime production. **Results:** Among 100 clinical isolates of staphylococcus aureus 82% of them showed beta hemolysis, 79% produces DNase, 72% produces slime. The proportion of MRSA amongst S. aureus isolates was found to be 53%. MSSA were 100% sensitive to vancomycin and linezolid 85.10% to Doxycycline, 82.98% to clindamycin, 72.34% to chloramphenicol, 68.09% Co- trimoxozole, 65.95% to erythromycin, 61.70% gentamicin, 51.06% to Ciprofloxacin, 12.77% topenicillin. MRSA were 100% sensitive to vancomycin and linezolid, 71.69% Doxycycline, 64.15% to Chloramphenicol, 60.37% to Clindamycin, 43.39% to Ciprofloxacin, 39.62% Gentamicin, 37.74% Co- trimoxozole, 35.84% to Erythromycin and all the isolates were resistant to penicillin. **Conclusion:** Understanding of virulence mechanisms and antibiotic susceptibility pattern of Staphylococcus aureus is important for effective management of infections.

Key words: Antibiotic sensitivity, D Nase test, Staphylococcus aureus, Slime production, Virulence factors.

Introduction

Staphylococcus aureus is one of the most prevalent and clinically significant pathogen, causing variety of infections ranging from mild skin and soft-tissue infections to serious life-threatening systemic infections and is a leading cause for hospital associated (HA) and community associated (CA) infections worldwide [1,2]. It causes skin, bone, urinary tract infections, soft tissue infections, pneumonia, bacteremia and other invasive infections in community and hospital settings [3].

Emergence of methicillin resistance among Staphylococcus aureus has reduced therapeutic alternatives available to treat staphylococcal infections [4]. Methicillin-resistant S. aureus (MRSA) infections account for 40-60% of all health care associated S. aureus infections in many centres across the world [1]. Report from the National Nosocomial Infections Surveillance System of the Centers for Disease Control and Prevention, showed that MRSA in India and USA accounts for > 60% of S. aureus isolates causing health care associated infection in ICUs. Drug-resistant strains limit the therapeutic options, creating an economic and social burden tothe healthcare system[3].

Virulence factors of S. aureusinclude slime formation, secreted enzymes, toxins like lipases, DNase, proteases, hemolysins and some super antigens like toxic shock syndrome toxin and enterotoxin [5]. The pathogenicity of S. aureus depends on various virulence factors associated with adherence, evasion of the immune system and damage of the host [6,7]. The presence of

Manuscript received: 24th February 2019
Reviewed: 4th March 2019
Author Corrected: 10th March 2019
Accepted for Publication: 15th March 2019

two or more virulence factors could increase the pathogenic ability of organism in relation to those that express only one virulence factor [8].

It is important for treating physicians to know the virulence factors and antibiotic susceptibility patterns of S. aureus isolates in their region as it shows differences among regions andit will help the clinician to choose appropriate antibiotic and to control the emergence of drug resistant strains.

Aims and objectives of the study

1. To isolate staphylococcus aureus from various clinical samples.
2. To study the virulence factors of Staphylococcus aureus. i. ebeta hemolysin, detection of DNase, detection of slime formation.
3. To study the antibiogram of Staphylococcus aureus and to detect methicillin resistance among them.

Materials & Methods

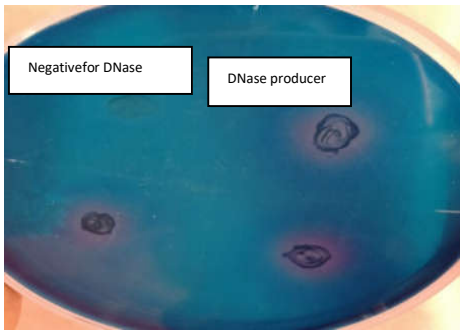
Place of study: Present study was done at Shimoga Institute of Medical Sciences, Shivamogga, for six month duration from January 2018 to June 2018.

Ethical consideration & permission: Study was done after obtaining institutional ethics committee clearance.

Statistical Methods: All Data were analysed and expressed in terms of percentage.

Collection of Samples and processing: The study was conducted on 100 staphylococcusaureus isolates from various clinical samples like blood, exudates, urine and respiratory samples received at Microbiology laboratory from Mc Gann teaching hospital, attached to Shimoga institute of medical sciences. These samples were processed on blood agar and Mac Conkey agar media and incubated at 37°C under aerobic conditions. The organisms were identified as per standard conventional methods [9].

Detection of DNase: This test was carried out by using DNase agar. The organisms were spot inoculated in DNase agar and incubated at 37^0C for 24 hours. Then flood the plate with 1N HCl. After standing a few minutes, examine the plate against a dark background. Clearing zone around the spotted colony is considered as positive for DNase [5,9,10].



Detection of beta hemolysin: Staphylococcus aureus were spot inoculated onto 5% sheep blood agar and incubated at 37^0 C overnight. After overnight incubation the plates were kept at 4^0c to observe hot –cold type of hemolysis produced by beta hemolysin [5].

Detection of Slime formation: Slime formation was detected using congo red agar (CRA) containing brain heart infusion broth, 5% sucrose, agar and 0.08% congo red. Strains were inoculated and plates were incubated at 37^0C

overnight. Dry black colonies of Staphylococcus aureus strains with crystalline consistency were considered as slime producers and those that produced pink colonies were considered as negative for slime formation [5,11].

Antibiotic susceptibility testing: Antimicrobial susceptibility testing of isolates was done on Mueller-Hinton agar by Kirby-Bauer disc diffusion method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. In the present study the susceptibility testing was carried out usingthe following antibiotics: Penicilline (P) Co-trimoxazole (COT), Clindamycin (CD), Erythromycin (E), Gentamicin (G), Chloramphenicol (c), Ciprofloxacin (CIP), Doxycycline (DO), Vancomycin (VA), Linezolid (LZ), Cefoxitin (cx)[12].

Detection of MRSA: Methicillin resistance was detected by disc diffusion method using 30µg cefoxitin disc which remains as a surrogate marker to identify mecA mediated resistance. The strains were considered as methicillin susceptible (MSSA-Methicillin susceptible staphylococcusaureus) if the zone of inhibition is ≥22mm and considered as MRSA (Methicillin resistant staphylococcusaureus) if the zone of inhibition was ≤21mm [12].

Results

Present study was carried out in the department of microbiology, Shimoga Institute of Medical Sciences, Shivamogga. The observations made from the study are shown in following tables.

Table-1: Staphylococcus aureus isolates obtained from various clinical specimens.

Different clinical specimens	Staphylococcus aureus isolates (%)
Exudate	69(69%)
Blood	21(21%)
Urine	6(6%)
Respiratory samples	4(4%)
Total	**100(100%)**

Among 100 clinical isolates of staphylococcus aureus 69% were from exudates samples, 21% from blood, 6% from urine, 4 from respiratory samples.

Table 2: Distribution of various virulence factors among staphylococcus aureus

Virulence factors	No of isolates N- 100	%
Beta hemolysin	82	82%
DNase production	79	79%
Slime production	72	72%

Among 100 clinical isolates of staphylococcusaureus 82% of staphylococcusaureus showed Beta hemolysis, 79% produced DNase, 72% were slime producers.

Table-3: Antibiotic sensitivity pattern of Staphylococcus aureus.

Antibiotics tested	Organisms isolated	
	MSSA N=47 (%)	MRSA N=53 (%)
Penicillin	6 (12.77)	0
Co-trimoxazole	32 (68.09)	20 (37.74)
Clindamycin	39 (82.98)	32(60.37)
Ciprofloxacin	24(51.06)	23(43.39)
Cefoxitin	47(100)	0
Chloramphenicol	34 (72.34)	34(64.15)
Doxycycline	40(85.10)	38(71.69)
Erythromycin	31 (65.95)	19 (35.84)
Gentamicin	29 (61.70)	21(39.62)
Linezolid	47 (100)	53(100)
Vancomycin	47(100)	53 (100)

The proportion of MRSA among Staphylococcusaureus isolates was found to be 53%. MSSA were 100% sensitive to vancomycin and linezolid, 85.10% to Doxycycline, 82.98% to clindamycin, 72.34% to chloramphenicol, 68.09% Co-trimoxozole, 65. 95% toerythromycin. 61.70% gentamicin, 51.06% to Ciprofloxacin,12.77% to penicillin. MRSA were 100% sensitive to vancomycin and linezolid, 71.69% Doxycycline, 64.15% to Chloramphenicol, 60.37% to Clindamycin, 43.39% to Ciprofloxacin, 39.62% Gentamicin, 37.74% Co- trimoxozole, 35.84% to Erythromycin and all the isolates were resistant to penicillin. The antibiotic sensitivity results showed that all MRSA isolates were significantly more resistant to antibiotics ascompared to MSSA isolates.

Discussion

MRSA is an important clinically significant pathogen, incidence of which is increasing every year. The organism has an ability to spread and cause outbreaks. Knowledge about virulence factors and prevalence of staphylococcus aureus and their current antimicrobial profile is necessary in the selection of appropriate empirical treatment for these infections, formulation of antibiotic policy, infection control, and patient management [13,4,14].

In our study, 79% of the staphylococcus aureusisolates were positive for DNase test. Another study by Kateete David P et al., reported 75% positivity for DNase test [15]. Study by Alice P. Selvabai et al. reported 86% positivity for the DNase test among MRSA isolates [5]. In the present study 82% of the Staphylococcus aureus isolates showed beta hemolysis. Study by Alice P. Selvabai et al. reported 67% of the MRSA isolates showed beta hemolysis [5]. In our study 72% of the staphylococcus aureus strains were found to be slime producers. Study by Adrianna Podbielskaet al. reported 69% of staphylococcus aureus were slime producer [11]. Study by Alice P. Selvabai et al. reported 61% were slime producers among MRSA isolates [5]. Slime producing strains of Staphylococcus aureus has the ability to form intact biofilm and also have higher rate of colonization in host tissues. Slime production is considered as a significant virulence factor for some strains of staphylococci [16]. The pathogenicity of S. aureus depends on various virulence factors associated with adherence, evasion of the immune system and damage of the host [6,7].

In our study the proportion of MRSA among Staphylococcus aureus isolates was 53%. MSSA were 100% sensitive to vancomycin and linezolid, 85.10% to Doxycycline, 82.98%to clindamycin, 72.34% to chloramphenicol, 68.09% Co-trimoxozole 65.95% to erythromycin. 61.70% gentamicin, 51.06% to Ciprofloxacin, 12.77% to penicillin.

MRSA were 100% sensitive to vancomycin and linezolid, 71.69% Doxycycline, 64.15% to Chloramphenicol, 60.37% to Clindamycin, 43.39% to Ciprofloxacin, 39.62% Gentamicin,, 37.74% Co-trimoxozole, 35.84% to Erythromycin and all the isolates are resistant to penicillin. Study done by Rajaduraipandiet alreported al most all clinical MRSA strains (99.6%) were resistant to penicillin, 93.6% to ampicillin and 63.2% towards gentamicin, co-

trimoxazole, cephalexin, erythromycin, and cephotaxime [13]. In a Study done by Gitau et al. A total of 944 S. aureus isolates were analyzed. High sensitivity of S. aureus was observed for quinupristin/ dalfopristin (100%), tigecycline (98.2), imipenem (98%), nitrofurantoin (97.6%), linezolid (97.3%), teicoplanin (97.1%) and vancomycin (95.1%). High resistance was recorded against penicillin G (91.9%), trimethoprim/ sulfamethoxazole (56.9%) and tetra-cycline (33.2%). MRSA prevalence was 27.8%. Both MRSA and MSSA were highly susceptible to quinupristin/ dalfopristin, tigecycline, linezolid, nitrofurantoin, ampicillin/ sulbactam and vancomycin and showed high resistance to commonly used antibiotics such as gentamycin, erythromycin, levofloxacin and tetracycline [3].

In a study done by Kitara LD et al. reported antibiotic susceptibility to Ampicillin (75.0%), Chloramphenicol (34.4%), Ciprofloxacin (1.6%), Erythromycin (7.8%), Gentamycin (0%), Methicillin (1.6%), Tetracycline (45.3%) and Co-trimoxazole (50.0%)[17]. Study by Alain C. Juayang et al evaluated the antimicrobial resistance of S. aureus isolated from clinical specimens and to put emphasis on the prevalence of MRSA and Inducible Clindamycin Resistance.

A total of 94 cases from 2010 to 2012 were diagnosed to have S. aureus infection using conventional bacteriologic methods. From these cases, 38 (40.6%) were identified as MRSA.

Wounds and abscesses were considered to be the most common specimens with MRSA infections having 71.05% while blood was the least with 5.3%. For drug susceptibility, out of the 94 S. aureus cases, including MRSA, 100% were susceptible to linezolid. It wasthen followed by tetracycline having a mean susceptibility of 95%; while penicillin G was ineffective with 94 cases having 0% susceptibility [18].

Study by Edet E. Udo et al showed All MRSA isolates were susceptible to linezolid, vancomycin, and teicoplanin. However, some isolates were resistant to kanamycin (2,979; 43%), ciprofloxacin (2,955; 42.7%), erythromycin and clindamycin (2,935; 42.4%), fusidic acid (2,858; 41.2%), gentamicin (2,665; 38.5%), tetracycline (2,652; 38.3%), and trimethoprim (2,324; 33.5%).

Whereas the prevalence of resistance to most antibiotics showed annual variations, those resistant to chloramphenicol and rifampicin increased from 2.6 and 0.1% to 9.6 and 1.6%, respectively, and high-level mupirocin resistance declined from 9.3% in 2011 to 3.6% in 2015 [19].

For the effective management of Staphylococcus aureus infection, knowledge about virulence factors and antibiotic sensitivity patters play a vital role. Conducting regular studies to know the changing trends of antibiotic sensitivity will help the treating physician to start empirical therapy.

Conclusion

MRSA is an important cause of healthcare-associated and community associated infections. It is important to do culture and sensitivity of specimens when Staphylococcus aureus infection is suspected. Continuing surveillance to assess the prevalence, geographic distribution, antibiogram, effective infection control measures are necessary to reduce the incidence of infections due to methicillin resistant staphylococcus aureus and it will also help the clinician to choose appropriate treatment options and to control the emergence of drug resistant strains.

What this study adds to existing knowledge? The publication of such surveillance study, data describing regional antimicrobial susceptibility/ resistance rates in clinical isolates of staphylococcus aureusis essential to stimulate antimicrobial stewardship efforts as well as to identify emerging resistance trends and geographic diversity over time.

Contribution by authors

- Study concept, Data collection, Manuscript writing, Data compiling, literature review: Dr. Vedavati B. I.
- Study concept, Manuscript writing: Mallikarjun koppad.
- Manuscript writing: Dr. D. E Premalatha.
- Data collection, Manuscript writing, Data compiling: Akshatha. Y. J.
- Manuscript editing, final review and approval: Dr Halesh L.H.

Funding: Nil; **Conflict of Interest**: None initiated
Permission from IRB: Yes

References

1. Fomda BA, Thokar MA, Khan A, et al. Nasal carriage of Methicillin-resistant Staphylococcus aureus among healthy population of Kashmir, India. Indian J Med Microbiol. 2014 Jan-Mar;32(1):39-43.doi: 10.4103 / 0255-0857.124296.

2. Perumal N, Dass BS, Mani S, et al. Prevalence of bi-component pore-forming toxin genotypes of Staphylococcus aureus causing skin and soft tissue infections. Indian J Med Microbiol. 2017 Jan-Mar; 35 (1): 146-147. doi: 10.4103/ijmm.IJMM_16_424.

3. Gitau et al. Antimicrobial susceptibility pattern of Staphylococcus aureus isolates from clinical specimens at Kenyatta National Hospital. BMC Res Notes (2018) 11:226. https://doi.org/10.1186/s13104-018-3337-2.

4. Deotale V, Mendiratta DK, Raut U, et al. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Indian J Med Microbiol. 2010 Apr-Jun;28(2):124-6. doi: 10.4103/ 0255-0857. 62488.

5. Alice P. Selvabai et al. Characterization of Methicillin Resistant Staphylococcus aureus based on its virulence factors and antimicrobial susceptibility profile. Indian J Microbiol Res 2017;4(1):74-78.

6. Alli OA, Ogbolu DO, Shittu AO, et al. Association of virulence genes with mecA gene in Staphylococcus aureus isolates from Tertiary Hospitals in Nigeria. Indian J Pathol Microbiol. 2015 Oct-Dec; 58 (4):464-71. doi: 10.4103/0377-4929.168875.

7. Justyna Bien et al. Characterization of Virulence Factors of Staphylococcus aureus: Novel Function of Known Virulence Factors That Are Implicated in Activation of Airway Epithelial Proinflammatory Response. Journal of Pathogens. Volume 2011, Article ID 601905, 13 pages doi:10.4061/2011/601905.

8. Ebrahimi A, Ghasemi M, Ghasemi B. Some Virulence Factors of Staphylococci Isolated From Wound and Skin Infections in Shahrekord, IR Iran. Jundishapur J Microbiol. 2014 Apr;7(4):e9225. doi: 10.5812/jjm.9225. Epub 2014 Apr 1.

9. Collee JG, Marr W. Culture of bacteria. Mackie and McCartney Practical medical microbiology. 14th edn. Edinburg: Churchill Livingstone.1996: 95- 260.

10. Tukyilmaz, et al. Determination of some Virulence Factors in Staphylococcus Spp. Isolated from Various Clinical Samples.Turk J Vet Anim Sci 30 (2006). 127-132

11. Adrianna Podbielska et al. Slime Production by Staphylococcus aureus and Staphylococcus epidermidis Strains Isolated from Patients with Diabetic Foot Ulcers. Arch. Immunol. Ther. Exp. (2010) 58:321–324. DOI: 10.1007/s00005-010-0079-9 · Source: PubMed.

12. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; 28th ed. Informational Supplement. CLSI document M100-S25. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

13. Rajaduraipandi K, Mani KR, Panneerselvam K, et al. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: a multicentre study. Indian J Med Microbiol. 2006 Jan; 24 (1):34-8.

14. Kale P, Dhawan B. The changing face of community-acquired methicillin-resistant Staphylococcus aureus. Indian J Med Microbiol. 2016 Jul-Sep; 34 (3):275-85. doi: 10.4103/0255-0857.188313.

15. Kateete et al. Identification of Staphylococcus aureus: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. Annals of Clinical Microbiology and Antimicrobials 2010, 9:23.http://www.ann-clinmicrob.com/content/9/1/23.

16. Arslan S, Ozkardes F. Slime production and antibiotic susceptibility in staphylococci isolated from clinical samples. Mem Inst Oswaldo Cruz. 2007 Feb; 102 (1):29-33.

17. Kitara LD, Anywar AD, Acullu D, et al. Antibiotic susceptibility of Staphylococcus aureus in suppurative lesions in Lacor Hospital, Uganda. Afr Health Sci. 2011 Aug;11 Suppl 1:S34-9.

18. Alain C. Juayang. Antibiotic Resistance Profiling of Staphylococcus aureus Isolated from Clinical Specimens in a Tertiary Hospital from 2010 to 2012. Interdisciplinary Perspectives on Infectious Diseases. Volume 2014, Article ID 898457, 4 pageshttp://dx.doi.org/ 10. 1155/2014/898457.

19. Udo EE, Boswihi SS. Antibiotic Resistance Trends in Methicillin-Resistant Staphylococcus aureus Isolated in Kuwait Hospitals: 2011-2015. Med Princ Pract. 2017; 26 (5): 485-490. doi: 10.1159/000481944. Epub 2017 Oct 4.

.....

How to cite this article?

Vedavati B. I, Halesh L.H, Koppad M, Premalatha D. E, Akshatha. Y. J. Study of the virulence factors and antimicrobial susceptibility profile of staphylococcus aureus isolated from clinical samples at tertiary care centre. Trop J Path Micro 2019;5(3):120-125.doi:10.17511/jopm.2019.i03.02.

.....