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Original Research Article

Isolation of *Staphylococcus aureus* in Purulent Infective Conditions with Special Reference to MRSA

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Abstract

Background: Staphylococcus aureus is a pathogen worldwide with large disease burden. Methicillin resistant staphylococcus aureus (MRSA) is prevalent in hospital care settings and community. Timely diagnosis and treatment is essential to avert further complications of this infection as compared to Methicillin sensitive Staphylococcus aureus (MSSA). Aim of the study was to determine the prevalence of Methicillin-resistant Staphylococcus aureus (MRSA) from pus samples in a tertiary care hospital and to analyze the antibiotic susceptibility patterns of MRSA isolates. ***Methodology***: A cross sectional study which was done in a tertiary care hospital from Jan 2018-June 2018. Various clinical specimens were cultured and staphylococcus aureus was isolated and identified using standard biochemical tests and CLSI guidelines. ***Results:*** Out of 1090 pus samples processed 597 were growth positive and among these 196 were gram positive. Out of 196 gram positive isolates 119 were staphylococcus aureus of which 56 were MRSA & 63 were MSSA. All MRSA isolates were sensitive to Vancomycin and Linezolid and moderate sensitivity to Gentamicin and Co-trimoxazole. ***Conclusion:*** In hospital setting MRSA infection cause of worry due to resistance to commonly prescribed drugs. Regular surveillance and robust Antimicrobial stewardship can help to limit these infections.

Key words: Pus, MRSA, Oxacillin.

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INTRODUCTION

Staphylococcus aureus is a versatile pathogen capable of colonizing humans and mammals, and causing skin and invasive diseases such as endocarditis, osteomyelitis, and severe sepsis [1]. Staphylococcus aureus, the major pathogen of human infectious diseases which can cause various lesions by secreting a variety of virulence factors, has been becoming a great problem at home and abroad [2]. The still worrying occurrence of methicillin-resistant Staphylococcus aureus (MRSA) in many parts of the world poses a major challenge to health care systems by increasing the burden of disease [3]. It has overcome most of the therapeutic agents that have been developed in the recent years and hence the antimicrobial chemotherapy for this species has always been empirical. Many of these MRSA isolates are becoming multidrug resistant and are susceptible only to glycopeptide antibiotics such as vancomycin [4]. Low level resistance even to vancomycin is emerging at present [5]. The prolonged hospital stay, indiscriminate use of antibiotics, lack of awareness, receipt of antibiotics before coming to the hospital etc. are the possible predisposing factors of MRSA emergence [6]. Serious endemic and epidemic MRSA infections occur globally as infected and colonized patients in hospitals mediate the dissemination of these isolates and hospital staff assists further transmission [7].The development of resistance to multiple antibiotics and control of disease transmission by MRSA isolates in hospitals/communities have been recognized as the major challenges as the bacterial population that expresses the resistance phenotype varies according to the environmental conditions. Having, the knowledge of prevalence of MRSA and their current antimicrobial profile become necessary in the selection of appropriate empirical treatment of these infections. We determined the prevalence of MRSA from different clinical pus samples and their in vitro susceptibility pattern to various antimicrobial agents to record the current status

of MRSA response to commonly used anti Staphylococcus antibiotics in our tertiary care hospital.

MATERIALS AND METHODS

A total 1090 pus samples were processed for Staphylococcus aureus screening. All the samples were aseptically handled and processed. All the samples were Gram stained to determine the likely organism present. Subsequently, the clinical pus samples from various sites were inoculated on to blood agar plates (aerobic with 5% CO2), MacConkey agar and incubated at 37ºC for 24 hours. The colonies of Gram-positive cocci in clusters were further confirmed. All strains were tested for catalase test, further tested for the production of free and bound coagulase enzyme using slide and tube coagulase test as shown in Fig:1&2based on standard methods[8]. Staphylococcus aureus ATTC-25923 of known coagulase production was included as control strain. All the confirmed S. aureus strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method using Oxacillin disc (1µg) obtained from Hi Media Laboratories Pvt. Ltd by Kirby Bauer disc diffusion method on Muller Hinton agar with 2% NaCl as shown in Fig: 3, then incubated at 37^0 C as per CLSI guidelines [9]. Plates were incubated overnight for 18-24 hours at 37^0C and then examined to confirm a confluent lawn of growth obtained. A zone size of ≤ 21 mm was reported as Methicillin resistant S. aureus (MRSA) and ≥22 mm was reported as Methicillin sensitive S.aureus (MSSA). The antibiotics used were Gentamicin (10µg), Erythromycin (15µg), Cotrimoxazole (25µg), Ciprofloxacin (5µg), Clindamycin (2µg), Vancomycin (30µg) and Linezolid (30µg) were tested. The results were formulated according to CLSI guidelines. S. aureus ATCC 25923 was used as reference strain for the standardization of antibiotic susceptibility testing.

Fig-1: Slide Coagulase Test

Fig-2: Tube Coagulase Test

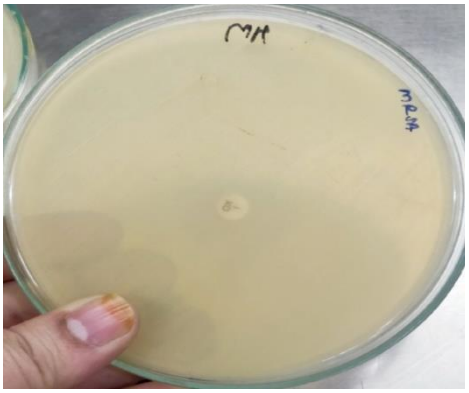


Fig-3: Oxacillin Disk Showing Resistance (MRSA)

RESULTS

During a period of 6 months a total number of 1090 pus samples were processed.Out of these 547 samples showed growth of pathogens, 196 samples were gram positive organisms followed by 334 gram negative. 17 of the pus samples had growth of yeast (Table 1). Among 196 Gram positive cocci, 119 Staphylococcus aureus were isolated (Table 2). Out of the119 Staphylococcus aureus reported 56(47.05%) strains were Methicillin Resistant Staphylococcus aureus (MRSA) and the remaining 63(62.94%) strains were methicillin sensitive Staphylococcus aureus (MSSA)(Table 3). Out of these 196 patients 68 were males and 128 were females with an age group between 16-50 years. Inducible clindamycin resistance was shown by 67.85% of MRSA isolates and 26.98% of MSSA isolates. In this study with maximum isolation of Staphylococcus aureus infection was from surgery ward followed by obstetrics/gynaecology, orthopaedics, medicine etc.

Table-1: Sample Distribution

Total Samples Received	**Samples with Growth**			**NO Growth**
1090	547			543
	Gram Positive	Gram Negative	Yeast	
	196	334	17	

Table-2: Gram Positive Organisms Distribution

Total Gram positive organisms isolated	**Staphylococcus aureus**	**Coagulase Negative Staphylococcus Spp.(CoNS)**	**Streptococcus pyogenes**	**Enterococcus spp.**	**Corynebacterium spp.**
196	119	72	2	1	2

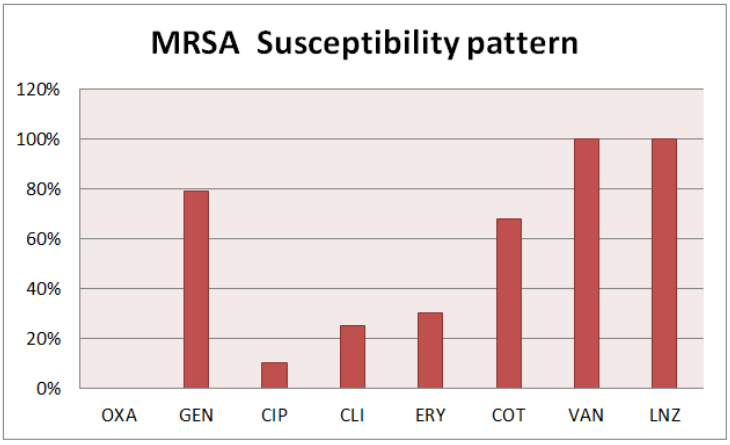
Table-3: Total MSSA and MRSA Isolated

Total Staphylococcus aureus(n=119)	
Methicillin Sensitive Staphylococcus aureus	Methicillin Resistant Staphylococcus aureus
56 (47.05%)	63 (52.94%)

Staphylococcus aureus was reported from samples obtained from various infective sites, most of these were from wound infections, post surgical site infections and abscess formation. MRSA was reported mostly from wound infection (Table4).

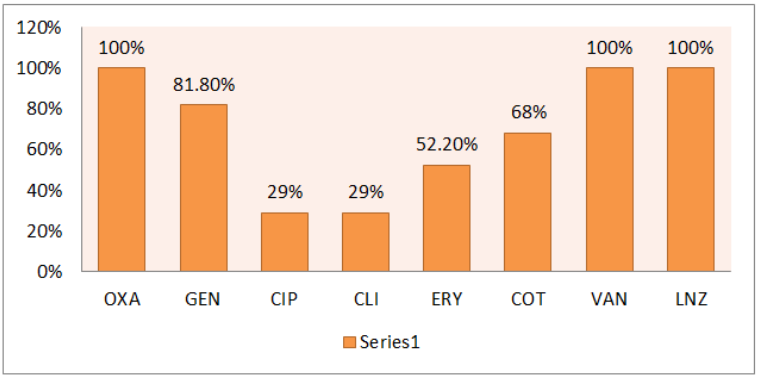
Table-4: MRSA and its association from various clinical conditions

Various Infective Conditions	No. of Staphylococcus aureus isolated	MRSA	MRSA percentage in the respective infection (%)
Wound infections	42	30	71.42
Foot cellulitis	18	9	50
Abcesses	35	9	25.71
Hernia	4	1	25
Sepsis	5	2	40
Osteomyelitis	2	1	50
Fistula in anowound	2	0	00
Appendicectomy wound	4	1	25
LSCS suture line infection	1	0	00
Femur implant wound	1	0	00
Varicocele wound	1	0	00
Ulcer	1	1	100
Lumbar disc space infection	1	0	00
Diabetic foot	1	1	100
Septic arthritis	1	1	100
TOTAL	119	56	47.05



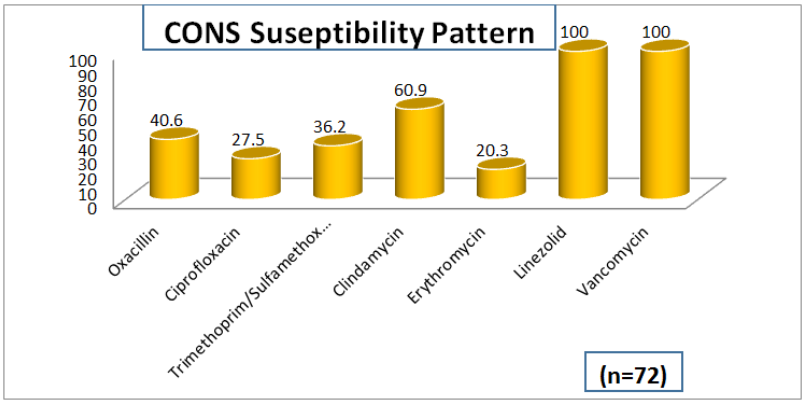
Graph-1: Graph showing MRSA antibiotic sensitivity pattern

All MRSA isolates were sensitive to vancomycin and linezolid (Graph1). Also, gentamicin and cotirmoxazole showed good sensitivity of approximately 80% and 70% respectively to these strains.



Graph-2: Graph showing MSSA antibiotic sensitivity pattern

All MSSA isolates were sensitive to vancomycin and linezolid (Graph2), whereas gentamicin and cotrimoxazole here too showed good sensitivity of 80% and 70% respectively. Erythromycin was also sensitive to 50% of the MSSA isolates. The coagulase-positive Staphylococcus aureus is the most important human pathogen in this genus but, Coagulase-negative Staphylococcus (CoNS) have been increasingly associated as opportunistic pathogens with serious nosocomial infections and it was the second most isolated gram positive organisms from our sample group.CoNS are part of normal skin flora and have emerged as important pathogen in hospital-acquired infections. However, these have been usually sensitive to commonly used antibiotics till a decade back. Emerging resistance is evident in these species and is reflected in isolates in our study too (Graph 3).



Graph-3: Graph showing CONS antibiotic sensitivity pattern

DISCUSSION

Staphylococcus aureus is capable of causing a variety of human infections, including fatal invasive and toxic conditions. It also possesses a differential ability to spread and cause hospital associated outbreaks of infection [10]. Resistance to multiple antibiotics among the Staphylococcus aureus isolates has been reported widely and has proved to be a major challenge in combating infections and in hospital infection control as well [11,12]. In this study high incidence of MRSA infection followed by Coagulase Negative Staphylococcus was seen. Co NS is common isolates from nonsterile body sites and has traditionally been described as skin commensals, with substantially less virulence than Staphylococcus aureus [13]. CoNS showed only 61% sensitivity to clindamycin and 36.5% and 27.5% for ciprofloxacin and cotrimoxazole respectively and only 20% towards erythromycin. But, vancomycin and linezolid had 100% sensitivity. Similar sensitivity towards vancomycin and linezolid was also reported by Singh L *et al.* [14]. In this study MRSA prevalence from pus samples was 47% which is much higher than most of the reports where it ranged between 20% to 32.8% [15, 16]. In present study, MRSA showed resistance to Erythromycin (70%), Clindamycin (75%), while vancomycin and linezolid showed 100% sensitivity followed by gentamicin (79%) sensitive and cotrimoxazole (68%) sensitive this was in accordance with a similar study conducted by Uwaezuoke and Ariratu[17]. Similarly, Rijal *et al*. [18] reported 96.9% susceptibility of Staphylococcus aureus isolates to vancomycin. However, increased use of these high-end drugs is not only expensive but also causes drug resistance to spread further. However, with appropriate antibiotic-sensitivity testing, other suitable antibiotics may be used in treatment of MRSA and use of vancomycin can be reserved for only severe, last-resort cases. Fluoroquinolones which had good antimicrobial activity against gram positive organisms, which were the hope for eradicating MRSA [19]. In the current study ciprofloxacin showed a resistance of 90%. This correlates with an earlier finding where it has been shown that the resistance to ciprofloxacin is steadily increasing from 39% in 1992 to 68% in 1996. In 1997 also a high incidence of ciprofloxacin resistance (95.8%) was reported [15]. So Ciprofloxacin should not be used as a 1st line antibiotic for the treatment of gram positive isolates. Erythromycin was 70% resistant which has also been reported in other studies [20, 21]. In this study MRSA isolates 38 (67.85%) showed erythromycin induced clindamycin resistance while 17 (26.98%) MSSA isolates showed erythromycin induced clindamycin resistance. Other studies also gave similar values and are in accordance with this study. For instance inducible resistance of 30% in MRSA and 10% in MSSA [22]. MRSA infections are treatable but it is important to prevent the spread of MRSA. An effective way to prevent such spread of Staphylococcus aureus and MRSA in hospital settings is to screen health care workers for the presence of this organism.

CONCLUSION

This study emphasizes the need for continuous monitoring of the antimicrobial susceptibility pattern of Staphylococcus aureus isolates, MRSA as well as MSSA for the selection of appropriate therapy. In addition, regular surveillance of hospital associated infections and implementation of a strict drug policy for antibiotics is the need of time.Also, due importance to be given to Co NS due to increased resistance being reported in these organisms too and clinicians should take cognizance about the hospitals antibiogram in this regards. Appropriate antimicrobials as well as their cost effectiveness need to be considered in drugs prescribed for staphylococcal infections. Good hospital infection control measures prove to be the main stay against these infections because antibiotics can never be an effective alternative to good medical practice.

REFERENCES

1. Orlin, I., Rokney, A., Onn, A., Glikman, D., & Peretz, A. (2017). Hospital clones of methicillin-resistant Staphylococcus aureus are carried by medical students even before healthcare exposure. *Antimicrobial Resistance & Infection Control*, *6*(1), 15.
2. Yu, Y., Yao, Y., Weng, Q., Li, J., Huang, J., Liao, Y., & Niu, J. (2017). Dissemination and molecular characterization of Staphylococcus aureus at a Tertiary Referral Hospital in Xiamen City, China. *BioMed research international*, *2017*.
3. Kriegeskorte, A., Idelevich, E. A., Schlattmann, A., Layer, F., Strommenger, B., Denis, O., ... & Becker, K. (2018). Comparison of different phenotypic approaches to screen and detect mecC-harboring methicillin-resistant Staphylococcus aureus. *Journal of clinical microbiology*, *56*(1), e00826-17.
4. Kshetry, A. O., Pant, N. D., Bhandari, R., Khatri, S., Shrestha, K. L., Upadhaya, S. K., ... & Raghubanshi, B. R. (2016). Minimum inhibitory concentration of vancomycin to methicillin resistant Staphylococcus aureus isolated from different clinical samples at a tertiary care hospital in Nepal. *Antimicrobial Resistance & Infection Control*, *5*(1), 27.
5. Samanta, D., & Elasri, M. O. (2014). The msaABCR operon regulates resistance in vancomycin-intermediate Staphylococcus aureus strains. *Antimicrobial agents and chemotherapy*, *58*(11), 6685-6695.
6. Anupurba, S., Sen, M. R., Nath, G., Sharma, B. M., Gulati, A. K., & Mohapatra, T. M. (2003). Prevalence of methicillin resistant Staphylococcus aureus in a tertiary referral hospital in eastern Uttar Pradesh. *Indian journal of medical microbiology*, *21*(1), 49.

7. McDonald, M. (1997). The epidemiology of methicillin-resistant Staphylococcus aureus surgical relevance 20 years on. *Australian and New Zealand journal of surgery*, *67*(10), 682-685.
8. Betty, A. F., Daniel, F. S., & Alice, S. W. (2002). Staphylococcus, Micrococcus and similar organisms, Chapter 19. *Baily and Scott's Diagnostic Microbiology, 11th edn.(Mosby Inc: St. Louis)*, 284.
9. Guideline, A. (2006). Clinical and Laboratory Standards Institute. *Wayne, PA*.
10. Nwankwo, E. O. K., & Shuaibu, S. A. (2010). Antibiotic susceptibility pattern of clinical isolates of pseudomonas aeruginosa in a tertiary health institution in kano, nigeria. *Journal of Medicine & Biomedical Sciences*, (4).
11. Majumder, D., Bordoloi, J. S., Phukan, A. C., & Mahanta, J. (2001). Antimicrobial susceptibility pattern among methicillin resistant Staphylococcus isolates in Assam. *Indian journal of medical microbiology*, *19*(3), 138.
12. Ikeagwu, I. J., Amadi, E. S., & Iroha, I. R. (2008). Antibiotic sensitivity pattern of Staphylococcus aureus in Abakaliki, Nigeria. *Pakistan Journal of Medical Sciences*, *24*(2), 231.
13. Bannerman, T. L. (2003). Staphylococci and other catalase positive cocci that grow aerobically. *Manual of clinical microbiology*, 384-404.
14. Singh, L., Cariappa, M. P., & Das, N. K. (2016). Drug sensitivity pattern of various Staphylococcus species isolated at a tertiary care hospital. *Medical Journal Armed Forces India*, *72*, S62-S66.
15. Shankar, C. U., Harish, B. N., Kumar, P. U., & Navaneeth, B. V. (1997). Prevalence of methicillin resistant Staphylococcus aureus in JIPMER Hospital-a preliminary report. *Indian Journal of Medical Microbiology*, *15*(3), 137.
16. Mehta, A. P., Rodrigues, C., Sheth, K., Jani, S., Hakimiyan, A., & Fazalbhoy, N. (1998). Control of methicillin resistant Staphylococcus aureus in a tertiary care centre: A five year study. *Indian Journal of Medical Microbiology*, *16*(1), 31.
17. Changchien, C. H., Chen, Y. Y., Chen, S. W., Chen, W. L., Tsay, J. G., & Chu, C. (2011). Retrospective study of necrotizing fasciitis and characterization of its associated Methicillin-resistant Staphylococcus aureus in Taiwan. *BMC infectious diseases*, *11*(1), 297.
18. Rijal, K. R., Pahari, N., Shrestha, B. K., Nepal, A. K., Paudel, B., Mahato, P., & Skalko-Basnet, N. (2008). Prevalence of methicillin resistant Staphylococcus aureus in school children of Pokhara. *Nepal med coll J*, *10*(3), 192-195.
19. David, M. Z., & Daum, R. S. (2010). Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic. *Clinical microbiology reviews*, *23*(3), 616-687.
20. Ellis, M. W., Griffith, M. E., Jorgensen, J. H., Hospenthal, D. R., Mende, K., & Patterson, J. E. (2009). Presence and molecular epidemiology of virulence factors in methicillin-resistant Staphylococcus aureus strains colonizing and infecting soldiers. *Journal of clinical microbiology*, *47*(4), 940-945.
21. Kazakova, S. V., Hageman, J. C., Matava, M., Srinivasan, A., Phelan, L., Garfinkel, B., & Dodson, D. (2005). A clone of methicillin-resistant Staphylococcus aureus among professional football players. *New England Journal of Medicine*, *352*(5), 468-475.
22. Prabhu, K., Rao, S., & Rao, V. (2011). Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. *Journal of laboratory physicians*, *3*(1), 25.