

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/mjafi

Short Communication

Drug sensitivity pattern of various *Staphylococcus* species isolated at a tertiary care hospital



Col Lavan Singh^{a,*}, Col M.P. Cariappa^b, N.K. Das^c

^a Senior Adviser (Pathology), Military Hospital Meerut, UP, India

^b Associate Professor, Dept of Community Medicine, Armed Forces Medical College, Pune 411040, India

^c Assistant Professor (Microbiology), Dr. D. Y. Patil Medical College, Pimpri, Pune, India

ARTICLE INFO

Article history:

Received 9 May 2015

Accepted 20 July 2016

Available online 17 October 2016

Keywords:

*Staphylococcus aureus*Coagulase-negative *Staphylococcus*

Antibiotic resistance

Macrolide–lincosamide–
streptogramin-B antibiotic
resistance

ABSTRACT

Background: *Staphylococcus aureus* and other *Staphylococcus* species are important pathogenic organisms and are responsible for various hospital infections. These are the predominant organisms found in pus and blood culture isolates. Infections arising due to these bacterial isolates are difficult to treat because of developing multidrug resistance.

Methods: Over a 1-year period at a tertiary care hospital laboratory, 524 *Staphylococcus* species were isolated from pus, blood and urine samples and species-level identification was done.

Results: *S. aureus* formed the predominant species (70.8%) followed by coagulase-negative *Staphylococcus* (CoNS) (29.20%). *S. aureus* (91%) was the main isolate from pus samples; however, CoNS was isolated in equally higher proportion in blood culture (63.58%). Among the CoNS, *Staphylococcus hemolyticus* was the main isolate (9.3%). β -Lactamase production, alteration of PBP and MLSB resistance were seen in variable degrees in different species.

Conclusion: CoNS group of *Staphylococcus* is becoming an important cause of infection at tertiary care centres. The increased multidrug resistance among various *Staphylococcus* species is a cause of great concern and requires adequate measures to prevent the spread of these microorganisms in the hospital and the community.

© 2016 Published by Elsevier B.V. on behalf of Director General, Armed Forces Medical Services.

Introduction

Staphylococcus are among the most important bacteria that cause diseases in humans. The coagulase-positive *Staphylococcus aureus* is the most important human pathogen in this

genus. Coagulase-negative *Staphylococcus* (CoNS) have increasingly been associated as opportunistic pathogens with serious nosocomial infections.¹ Isolates of *S. aureus* that have become resistant to methicillin are known as methicillin-resistant *S. aureus* (MRSA). The infection acquired by persons who have neither been hospitalized nor undergone any medical

* Corresponding author.

E-mail address: lsvaghuvanshi@yahoo.co.in (L. Singh).

<http://dx.doi.org/10.1016/j.mjafi.2016.07.009>

0377-1237/© 2016 Published by Elsevier B.V. on behalf of Director General, Armed Forces Medical Services.

procedure is referred to as 'Community-acquired' (CA-MRSA), and when acquired in the hospitalized patient, it is referred as hospital-acquired (HA-MRSA).²

Antimicrobial therapy is vital to the management of patients having staphylococcal infections. Antibiotic sensitivity pattern of such clinical isolates is becoming unpredictable and requires testing as a guide to therapy.³ Since the 1990s, CA-MRSA infections have been reported from various countries. CoNS are part of normal skin flora and have emerged as important pathogens in hospital-acquired infections. However, these have been usually sensitive to commonly used antibiotics till a decade back.

Due to increasing resistance to antimicrobial agents among Staphylococci, renewed interest has emerged to the use of macrolide (erythromycin, clarithromycin, roxithromycin and azithromycin), lincosamide (clindamycin and lincomycin) and streptogramins (streptogramin A – pristinamycin and dalfo-prisin; and streptogramin B – quinupristin). However, development of macrolide resistance worldwide has limited the use of these antibiotics. Macrolide resistance occurs either through target site modification (MLS_B phenotype, encoded by erm genes), efflux pump mechanism (MS phenotype, i.e. resistant to macrolide and streptogramins but sensitive to lincosamide, encoded by msrA/B genes) or decreased cell wall permeability. By the use of 'D test', inducible (iMLS_B) and constitutive (cMLS_B) can be differentiated. When the 'D test' is positive, it is iMLS_B, and when the resistance is both towards clindamycin and to erythromycin, it is cMLS_B. In vitro antibiotic sensitivity tests normally done in the laboratory cannot detect inducible resistance unless the 'D test' is done. Constitutive resistance to MLS antibiotics is confirmed by using molecular methods for the concerned erm genes. The msrA/B gene, first identified in *Staphylococcus epidermidis*, confers the so-called MS phenotype as ascribed to earlier. The msrA/B genes may be found in *Staphylococcus aureus* but are more common in CoNS.

On admission to hospital and treatment with antibiotics, patients often become colonised with more drug-resistant *S. aureus* and CoNS. In the recent past, CoNS has gained more clinical significance as they have been isolated in more numbers from patients having various risk factors, e.g. use of various intravascular catheters, prosthetic devices, foreign body implants, use of immunosuppressive drugs for renal transplant recipients and immunocompromised patients on chemotherapeutic agents. These infections are difficult to treat because of the underlying risk factors and increased drug resistance among CoNS species. The present study was carried out to identify the frequency of various *Staphylococcus* species and their current antibiogram pattern.

Materials and methods

A total of 524 isolates of Staphylococci were collected from pus, blood, urine and other miscellaneous samples including body fluids and sputum over a 1-year period from November 2012 to October 2013 at the laboratory of a tertiary care hospital and studied for antibiotic sensitivity patterns. The isolates were considered relevant when isolated in pure culture from infected sites. These isolates were initially identified by colony morphology, Gram staining, catalase, slide and tube coagulase

tests and anaerobic acid formation from mannitol.³ Further identification of different species of CoNS and antibiotic susceptibility was done by VITEK2 system (Biomerieux). The system identified beta-lactamases and PBP2 resistance. Kirby-Bauer disc diffusion method uses panel of required antibiotics as per CLSI (2013) guidelines that were placed in parallel.⁴ Discs contained the following antibiotics at specific absolute concentrations – penicillin (10 µg), oxacillin (20 µg), linezolid (30 µg), cefoxitin (30 µg), augmentin (20/10 µg), clindamycin (2 µg), erythromycin (15 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), tetracycline (30 µg) and gentamicin (10 µg).

Data were generated for β-lactamase production, modification of PBP2 and MLS_B resistance (both inducible and constitutive). The phenotypic method was used for our study by using erythromycin (15 µg) and clindamycin (2 µg) discs, kept 15 mm apart, for inducible type of MLS_B resistance. Isolates were considered having inducible resistance to clindamycin when showing flattening of clindamycin sensitivity zone adjacent to erythromycin sensitivity zone. Novobiocin resistance tests were also placed for quality control and confirmation. Controls ATCC 25923 for MRSA and 29213 for MSSA were used. Only phenotypic methods have been used in our study to distinguish the different isolates of *Staphylococcus aureus* (MRSA and MSSA) and coagulase-negative Staphylococci (CoNS).

Results

S. aureus was the single most common isolate obtained from all the clinical samples (70.8%) and was the predominant isolate from pus (91.8%). CoNS were the predominant microorganisms from blood samples (63.58%). Among the 10 identified CoNS species, *S. hemolyticus* was the most frequently isolated (9.3%), followed by *S. epidermidis* (8%) and *S. saprophyticus* (3.6%). From blood samples, *S. hemolyticus* (19.6%) and *S. epidermidis* (17.8%) were the main isolates, followed by *S. hominis* (6.08%) and *S. saprophyticus* (5.4%).

From urine, the main CoNS isolates were *S. saprophyticus* (22.8%) and *S. epidermidis* (20%). Detailed breakdown of isolates is given in Table 1. Antibiotic susceptibility testing showed varying degree of resistance by different staphylococcal species (Table 2). Maximum resistance was observed to

Table 1 – Species-wise distribution of different *Staphylococcus* isolates.

Species	Pus	Blood	Urine	Misc	Total
<i>S. aureus</i>	280	54	21	16	371
<i>S. hemolyticus</i>	11	29	01	08	49
<i>S. epidermidis</i>	06	26	08	02	42
<i>S. saprophyticus</i>	01	08	09	01	19
<i>S. hominis</i>	02	09	0	02	13
<i>S. xylosum</i>	03	05	0	0	08
<i>S. warneri</i>	0	04	01	02	07
<i>S. scuri</i>	01	03	0	0	04
<i>S. capitis</i>	0	04	0	0	04
<i>S. lentis</i>	0	04	0	0	04
<i>S. lugdunensis</i>	01	02	0	0	03
Total	305	148	40	31	524

Table 2 – Antibiotic resistance pattern (%) of main *Staphylococcus* species.

Species	P	Ox	Aug	Clind	E	Cip	Levo	Lz	Tet	Tg	Genta	Cx
<i>S. aureus</i> (371)	296 (80%)	196 (53%)	201 (54%)	92 (25%)	92 (25%)	316 (85%)	86 (23%)	0 (0%)	15 (4%)	0 (0%)	119 (32%)	196 (53%)
<i>S. hemolyticus</i> (49)	49 (100%)	49 (100%)	44 (90%)	8 (17%)	8 (17%)	41 (84%)	41 (84%)	0 (0%)	2 (4%)	0 (0%)	41 (84%)	49 (100%)
<i>S. epidermidis</i> (42)	37 (88%)	34 (81%)	33 (79%)	32 (77%)	32 (77%)	19 (45%)	8 (19%)	0 (0%)	0 (0%)	0 (0%)	12 (27%)	34 (81%)
<i>S. saprophyticus</i> (19)	14 (74%)	7 (37%)	14 (74%)	8 (42%)	7 (37%)	9 (48%)	3 (16%)	0 (0%)	4 (22%)	3 (16%)	8 (42%)	7 (37%)
<i>S. hominis</i> (13)	9 (69%)	9 (69%)	10 (77%)	10 (77%)	11 (85%)	5 (38%)	5 (38%)	9 (69%)	3 (23%)	1 (8%)	0 (0%)	9 (69%)
<i>S. xylosum</i> 2 (8)	2 (25%)	2 (25%)	2 (25%)	2 (25%)	0 (0%)	0 (0%)	0 (0%)	1 (0%)	1 (13%)	0 (0%)	0 (0%)	2 (25%)
<i>S. warneri</i> (7)	5 (72%)	4 (57%)	4 (57%)	2 (29%)	1 (14%)	1 (14%)	1 (14%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (57%)
<i>S. lentis</i> (4)	2 (50%)	1 (25%)	2 (50%)	3 (75%)	0 (0%)	1 (25%)	1 (25%)	1 (25%)	1 (25%)	1 (25%)	1 (25%)	3 (75%)
<i>S. capitis</i> (4)	1 (25%)	1 (25%)	1 (25%)	1 (25%)	2 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (25%)	1 (25%)
<i>S. sciuri</i> (4)	2 (50%)	2 (50%)	2 (50%)	2 (50%)	2 (50%)	2 (50%)	2 (50%)	1 (25%)	0 (0%)	0 (0%)	0 (0%)	2 (50%)
<i>S. lugdunensis</i> (3)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

P, penicillin; Ox, oxacillin; Aug, augmentin; Clind, clindamycin; E, erythromycin; Cip, ciprofloxacin; Levo, levofloxacin; Lz, linezolid; Tet, tetracycline; Tg, tigecycline; Genta, gentamicin; Cx, ceftioxin.

Note: All percentages refer to % resistance. The total number of isolates is given below the name of the organism.

penicillin, oxacillin and ciprofloxacin. *S. aureus* showed higher resistance to penicillin (80%), oxacillin (53%), augmentin (54%) and ciprofloxacin (85%). Thus, there were 153 CoNS isolates, and out of 371 isolates of *S. aureus*, 196 were MRSA (53%) and 175 were MSSA (47%). *S. hemolyticus* showed maximum resistance to penicillin (100%), oxacillin (100%) and augmentin

(90%). All the strains of *S. hemolyticus* (100%) were β -lactamase producers, followed by *S. epidermidis* (89%) and *S. aureus* (80%). Resistance to MLS_B (inducible) resistance was observed in 17.74% of the staphylococcal isolates and 10.30% had the MLS_B (constitutive) type. *S. aureus* had maximum resistance at 19% (Table 3).

Table 3 – Species-wise drug resistance pattern in different *Staphylococcus* isolates.

Species	β -Lactamase	PBP (mecA)/modification of PBP	iMLS _B	cMLS _B
<i>S. aureus</i> (371)	296 (80%)	196 (53%)	70 (19%)	22 (6%)
<i>S. hemolyticus</i> (49)	49 (100%)	49 (100%)	02 (4.0%)	06 (12%)
<i>S. epidermidis</i> (42)	37 (89%)	34 (82%)	16 (16%)	16 (16%)
<i>S. saprophyticus</i> (19)	14 (75%)	07 (36%)	02 (10%)	06 (31%)
<i>S. hominis</i> (13)	09 (69%)	09 (69%)	02 (15%)	01 (8%)
<i>S. xylosum</i> (8)	02 (25%)	02 (25%)	0	02 (25%)
<i>S. warneri</i> (7)	05 (71%)	04 (57%)	0	01 (14%)
<i>S. lentis</i> (4)	02 (50%)	01 (25%)	0	0
<i>S. capitis</i> (4)	01 (25%)	01 (25%)	01 (25%)	0
<i>S. sciuri</i> (4)	02 (50%)	01 (25%)	0	0
<i>S. lugdunensis</i> (3)	0	0	0	0

PBP, penicillin-binding protein; MLS_B, macrolide-lincosamide-streptogramin B; iMLS_B, Inducible MLS_B; cMLS_B, constitutive MLS_B. The total number of isolates is given below the name of the organism.

Discussion

S. aureus is the most commonly isolated bacterial pathogen and may be considered to be an emerging epidemic.⁵ In our study, out of 524 isolates, *S. aureus* was the commonest organism, while CoNS constituted nearly one-third of the total isolates. Among the CoNS, *S. hemolyticus* was the commonest isolate followed by other species as given in Table 1. The most frequently isolated species of CoNS in past studies were *S. epidermidis* (up to 80%) and *S. saprophyticus* (15.60%).⁶⁻⁸ 15% *S. hemolyticus* was isolated in another study.⁹ Frequency of different species varies considerably in different clinical samples, which is coherent with other studies.^{10,11}

In our study, maximum resistance to penicillin (79.58%) and oxacillin (58%) was observed. *S. aureus* showed 80% resistance against penicillin and 53% resistance against oxacillin. CoNS had similar resistance against penicillin (79%), but much higher resistance against oxacillin (71%). Older studies have shown more than 80% of CoNS isolates being resistant to methicillin and semisynthetic penicillins, and among them, *S. hemolyticus* showed maximum (100%) resistance to penicillin and oxacillin.¹⁰⁻¹² In our study, *S. hominis* and *S. epidermidis* showed higher resistance to erythromycin (85% and 77%). Both these isolates also showed higher resistance to clindamycin (77%) similar to another study done elsewhere.⁹ *S. hominis*, *S. lentis* and *S. sciuri* showed resistance to linezolid (69%, 25% and 25%). In our study, *S. aureus*, *S. hemolyticus* and *S. epidermidis* showed higher resistance to ciprofloxacin (85%, 84% and 45%) as compared to levofloxacin (23%, 84% and 19%), while lower resistance was observed elsewhere.⁷ *S. lugdunensis* did not show any drug resistance. Higher sensitivity was seen to vancomycin (100%) followed by linezolid, which again is coherent with other studies.^{10,11} *S. hemolyticus* showed maximum multidrug resistance to all the commonly used antibiotics.

Production of β -lactamase remains the single most common mechanism of drug resistance followed by modification of penicillin-binding proteins. Both these mechanisms are seen in all species in variable frequency except *S. lugdunensis* (Table 3). iMLS_B type resistance is seen more frequently (17.74%) than cMLS_B type (10.30%), but less frequently than the previous two mechanisms. *S. xylosus* and *S. warneri* showed resistance only of cMLS_B type.

Most frequently isolated species of CoNS that were isolated from clinical samples in past studies are *S. epidermidis* (up to 80%) and *S. saprophyticus* (15.60%).⁶⁻⁸ Among CoNS, *S. hemolyticus* remained the commonest isolate (32%) followed by *S. epidermidis* (27.45%) in our study; 15% *S. hemolyticus* was reported in another study.⁹ *S. hominis* (8.4%), *S. xylosus* (5.2%) and *S. warneri* (4.5%) were considered as important isolates in our study, with *S. lugdunensis* having the least frequency (1.9%). This is much lower as compared with another study.⁹ It appears that frequency of different species varies on different locations and hospitals. *S. saprophyticus* was predominantly isolated from urine samples and this frequency is coherent with other studies.^{10,11}

We observed maximum resistance to penicillin and oxacillin. Older studies have shown more than 80% of CoNS

isolates being resistant to methicillin and semisynthetic penicillins, and among them, *S. hemolyticus* showed maximum resistance to penicillin and oxacillin (up to 100%).¹⁰⁻¹² *S. hominis* and *S. epidermidis* showed higher resistance to erythromycin (85% and 77%). Both these isolates also showed higher resistance to clindamycin (77%) similar to another study done elsewhere.⁹ *S. hominis*, *S. lentis* and *S. sciuri* showed resistance to linezolid (69%, 25% and 25%). *S. aureus*, *S. hemolyticus* and *S. epidermidis* showed higher resistance to ciprofloxacin (85%, 84% and 45%) as compared to levofloxacin (23%, 84% and 19%) in our study, while lower resistance was observed elsewhere.⁷ *S. lugdunensis* did not show any drug resistance. Higher sensitivity was seen to vancomycin followed by linezolid, which again is coherent with other studies.^{10,11}

Production of β -lactamase remains the single most common mechanism of drug resistance followed by modification of penicillin-binding proteins. Both these mechanisms are seen in all species in variable frequency except *S. lugdunensis* (Table 3). iMLS_B (17.74%) type resistance is seen more frequently than cMLS_B type (10.30%), but less frequently than the previous two mechanisms. *S. xylosus* and *S. warneri* showed resistance only of cMLS_B type. Our study further re-emphasises that the results of the double disc diffusion tests do correlate well with fully automated systems. Fiebelkorn et al. described this reliable method (double disc diffusion test) for detecting inducible resistance to clindamycin in erythromycin-resistant isolates of *S. aureus* and CoNS.¹²

We suggest that a simple test for iMLS_B can be done by any laboratory to rule out inducible lincosamide resistance by carrying out the 'D' test. CoNS must be looked into carefully so that there are no treatment failures, and thus unnecessary clindamycin usage can be avoided as recommended elsewhere as well.^{4,13} Where molecular tests can be done, the corresponding genes for constitutive resistance can also be mapped in selected cases. By adopting such an approach, clinicians with the support of microbiologists, through correct identification and an early alert for resistance to MLS_B antibiotics, can help in reducing morbidity and perhaps, saving lives.

Conclusion

We conclude that *S. aureus* remains the most common pathogenic organism among all *Staphylococcus* species and CoNS are replacing *S. aureus* in blood and urine specimens. The frequency of CoNS species varies in different locations, regions and hospitals. In the present study, majority of CoNS belonged to *S. hemolyticus* and *S. epidermidis*, and this increase in frequency of *S. hemolyticus* could be the result of antibiotic pressure. The isolation of *S. epidermidis* from blood culture should be correlated well clinically and preferably from paired samples. There is a need for speciation of CoNS and proper study of their antibiogram for more effective patient care. Clinicians may take cognizance of the recommendation for local susceptibility patterns to be reviewed periodically when choosing the appropriate antibiotic in managing their patients.

Conflicts of interest

The authors have none to declare.

REFERENCES

1. Direct contact diseases. Willey JM, ed. In: *Prescotts Microbiology* 9th ed. New York: McGraw Hill; 2014:909-921.
2. Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA*. 2007;298(October (15)):1763-1771.
3. *Staphylococcus*, *Micrococcus* and similar organisms. Tille PM, ed. In: *Bailey and Scott's Diagnostic Microbiology* 13th ed. Elsevier; 2013:232-246.
4. Clinical and Laboratory Standards Institute Guidelines. <http://www.microbiolab-bg.com/CLSI.pdf> [accessed 12.04.15].
5. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev*. 2010;23:616-687.
6. Boucher HW, Corey GR. Epidemiology of methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis*. 2008;46(suppl 5):S344-S349.
7. Mohan U, Jindal N, Aggarwal P. Species distribution and antibiotic sensitivity pattern of coagulase negative *Staphylococci* isolated from various clinical specimens. *Indian J Med Microbiol*. 2002;20:45-46.
8. Singh R, Dhawan S, Moanty S, et al. Species distribution and antimicrobial susceptibility of coagulase negative *Staphylococci* in tertiary care hospital. *Indian J Med Res*. 2006;123:569-570.
9. Sheikh AF, Mehdinejad M. Identification and determination of coagulase-negative *Staphylococci* species and antimicrobial susceptibility pattern of isolates from clinical specimens. *Afr J Microbiol Res*. 2012;6(8):1669-1674.
10. Singh S, Banerjee G. Simple methods for speciation of clinically significant coagulase negative *Staphylococci* and its antibiotic sensitivity/resistant pattern in ICU of tertiary care centre. *Biomed Res*. 2008;19(2):97-101.
11. Asangi SY, Mariraj J, Sathyanarayan MS, Nagabhushan R. Speciation of clinically significant coagulase negative *Staphylococci* and their antibiotic resistant patterns in a tertiary care hospital. *Int J Biol Med Res*. 2011;2(3):735-739.
12. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative *Staphylococci*. *J Clin Microbiol*. 2003;41(10):4740-4744.
13. Prabhu K, Rao S, Rao V. Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. *J Lab Phys*. 2011;3(1):25-27.