

Clinically significant Coagulase Negative Staphylococci and their antibiotic resistance pattern in a tertiary care hospital

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Abstract

Objectives: To identify various species of coagulase negative staphylococci involved in neonatal septicaemia and determine their antimicrobial resistance pattern.

Methods: The prospective descriptive study was carried out from January 2012 to October 2013, at Umm Al-Qura University, Makkah, Saudi Arabia, and comprised clinical isolates of coagulase negative staphylococci recovered from the blood of neonates at Maternity and Children Hospital, Makkah.. The identification of species and antibiotic sensitivity for each isolate was done using Microscan Walk Away system.

Results: Of the 190 clinical isolates *S.epidermidis* was the most common species found 144(75.8%). The overall drug resistance among the species ranged from 1.6% to 99.5% to all the drugs tested, except to vancomycin and linezolid which were 100% sensitive. The highest drug resistance was exhibited by penicillin 189(99.5%), ampicillin 188(99%), oxacillin 178(93.6%) and augmentin 177(93%). The minimum drug resistance was shown by synercid 4(2.2%) and daptomycin 3(1.6%). All species were 100% resistant to penicillin and ampicillin, except *S.hyicus* and one isolate of *S.hominis-hominis* which was sensitive to ampicillin.

Conclusion: High rates of antibiotic resistance was seen among coagulase negative staphylococci to commonly-used antibiotics and majority of them were methicillin-resistant. The newer drugs tested can be used as an alternative.

Keywords: Coagulase, Staphylococcal infections, Drug resistance, Tertiary healthcare. (JPMA 64: 1171; 2014)

Introduction

Coagulase-negative Staphylococci (CoNS) are major cause of hospital-associated bloodstream infections and responsible for high morbidity and mortality, mainly in hospitalised patients and usually show multi-drug resistant (MDR) profiles.^{1,2} According to the Centre for Disease Control and Prevention National Healthcare Safety Network, CoNS has contributed to over 30% of hospital-wide central-line associated blood stream infections.³ There was change in the trend of causative agents of nosocomial infections during 1970 through 2000 from gram-negative bacteria to gram-positive bacteria. Due to this epidemiological change, CoNS were isolated as the most common organism from various settings of the hospital, including neonatal and paediatric patients.⁴

For the last 20 years CoNS has been identified as a major cause of neonatal septicaemia in neonatal intensive care units (NICU) and continue to be the most commonly isolated organisms in late-onset sepsis in NICU, particularly in low birth weight (LBW) neonates.^{5,6} CoNS were also identified as

the most common organisms in the blood cultures of newborns in NICUs, causing bacteraemia in 40-60% cases.⁷

CoNS are often resistant to multiple antibiotics and because of high rates of methicillin-resistance, β -lactam antibiotics are not the first drugs of choice. A worldwide SENTRY study showed that irrespective of geographical locations, nowadays about 70-75% of CoNS are resistant to methicillin.⁸

The increased recognition of pathogenic potential of CoNS and emergence of drug resistance among them warrants the need to identify various species of CoNS and determine their antibiotic resistance pattern. Hence, this study was undertaken to identify various species of CoNS isolated from blood culture of neonates, and to determine their antibiotic resistance pattern.

Material and Methods

The prospective descriptive study was carried out from January 2012 to October 2013, at the Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Umm Al-Qura University, Makkah, Saudi Arabia, and comprised clinical isolates of coagulase negative staphylococci recovered from the blood of neonates at Maternity and Children Hospital, Makkah, after getting approval from the institutional Bioethical Committee. The organisms were considered clinically significant when isolated in pure culture from blood which coincided with

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clinical signs and symptoms that included temperature instability, hypoxaemia, apnoea, bradycardia, lethargy and feeding intolerance.^{9,10}

The identification of CoNS species and the sensitivity pattern for each isolate was performed using Microscan Walk Away system (40SI, Siemens). The Microscan gram-positive identification and susceptibility panel (PBC-28) was used. The Microscan microtiter plate for gram-positive panel contained separate wells for biochemical agents for identification and separate wells in the same plate for antimicrobial agents with different concentration in double dilutions for sensitivity testing. The test was performed by touching freshly-grown five colonies of the test organism by using specific prompts for the purpose. These colonies were suspended in 25ml of pluronic suspension fluid. This inoculated fluid was dispensed in special trays and transferred to dehydrated substrates in the microtiter plate by RENOK system. The inoculated plates were then placed in the Microscan Walk Away system for identification and antibiotic sensitivity testing. The results were read automatically between 16-24 hours. The data of all isolates was entered and analysed using Microsoft Excel 2007.

Results

Of the 190 clinical isolates of CoNS that were identified up to species level in the study, *S.epidermidis* was the most common species found 144(75.8%), followed by *S.haemolyticus* 21(11.1%) and *S.hominis-homin* 16(8.4%) and others (Table-1).

The overall drug resistance among CoNS species ranged from 1.6% to 99.5% to all the drugs tested, except to vancomycin and linezolid which were 100% sensitive (Table-2). The highest drug resistance was exhibited by penicillin 189(99.5%), followed by ampicillin 188(99%), oxacillin 178(93.6%) and augmentin 177(93%), and others. The minimum drug resistance was seen in synercid 4(2.2%) and

Table-1: CoNS species identified at Maternity and Children hospital, Makkah.

CoNS Species	No (%)
<i>S.epidermidis</i>	144 (75.8)
<i>S.haemolyticus</i>	21 (11.1)
<i>S.hominis-homin</i>	16 (8.4)
<i>S.hominis-novo</i>	2 (1.1)
<i>S.warneri</i>	2 (1.1)
<i>S.hominis</i>	1 (0.5)
<i>S.capitis</i>	1 (0.5)
<i>S.hyicus</i>	1 (0.5)
<i>S.lugdunensis</i>	1 (0.5)
<i>S.auricularis</i>	1 (0.5)
Total	190 (100)

CoNS: Coagulase negative staphylococci.

daptomycin 3(1.6%). All CoNS species were 100% resistant to penicillin and ampicillin, except *S.hyicus* and one isolate of *S.hominis-homin* which was sensitive to ampicillin.

Apart from *S.epidermidis*, *S.haemolyticus* and *S.hominis-homin*, all other species were less frequently isolated (1-2 strains each). All these species showed almost 100% resistance to all the drugs tested except to vancomycin, synercid, daptomycin and linezolid. Two (100%) strains of *S.hominis-novo* (100%) and 1(6.25%) strain of *S.hominis-homin* were even resistant to daptomycin, and 1(100%) strain of *S.capitis* was resistant to synercid.

The drug resistance pattern for the first three CoNS species i.e., *S.epidermidis*, *S.haemolyticus* and *S.hominis-homin* was variable for other drugs tested (other than penicillin and ampicillin). All the three species exhibited high resistance to augmentin; *S.epidermidis* 137(95%), *S.haemolyticus* 20(95%) and *S.hominis-homin* 14(87%). While *S.auricularis*, *S.hyicus* and *S.lugdunensis* were 100% sensitive to augmentin. *S.epidermidis* showed equal resistance 117(81% each) both

Table-2: Antibiotic resistance pattern of different CoNS species.

CoNS Species	Resistance No (%)												
	No.	Ampicillin	Augmentin	Azithromycin	Clindamycin	Daptomycin	Erythromycin	Gentamicin	Linezolid	Oxacillin	Penicillin	Synercid	Vancomycin
<i>S.epidermidis</i>	144	144 (100)	137 (95)	117 (81)	94 (65)	0 (0)	117 (81)	123 (85)	0 (0)	137 (95)	144 (100)	3 (2)	0 (0)
<i>S.haemolyticus</i>	21	21 (100)	20 (95)	18 (86)	9 (43)	0 (0)	18 (86)	19 (90)	0 (0)	20 (95)	21 (100)	0 (0)	0 (0)
<i>S.hominis-homin</i>	16	15 (94)	14 (87)	8 (50)	8 (50)	1 (6)	8 (50)	8 (50)	0 (0)	15 (94)	16 (100)	0 (0)	0 (0)
<i>S.hominis-novo</i>	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)
<i>S.warneri</i>	2	2 (100)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)
<i>S.hominis</i>	1	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
<i>S.capitis</i>	1	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)
<i>S.hyicus</i>	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>S.lugdunensis</i>	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
<i>S.auricularis</i>	1	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)
Total	190	188 (99)	177 (93)	148 (78)	114 (60)	3 (1.6)	148 (78)	156 (82)	0 (0)	178 (93.6)	189 (99.5)	4 (2.2)	0 (0)

CoNS: Coagulase negative staphylococci.

to azithromycin and erythromycin, and *S.haemolyticus* showed 18(86%) resistance each to these two drugs. Similarly, *S.hominis-homin* showed equal resistance 8(50%) to azithromycin and erythromycin. *S.hominis-homin* also showed 8(50%) resistance each to clindamycin and gentamicin. The resistance to clindamycin was 94(65%) for *S.epidermidis* and 9(44%) for *S.haemolyticus*. However, both species showed high resistance to gentamicin: *S.epidermidis* 123(85%) and *S.haemolyticus* 19(90%).

Majority of the CoNS isolates 178(93.6%) were resistant to oxacillin. Less frequently isolated CoNS species showed 100% resistance to oxacillin, whereas *S.epidermidis* 137(95%), *S.haemolyticus* 20(95%) and *S.hominis-homin* 15(94%) were resistant to oxacillin.

Among the newer drugs tested (e.g., linezolid, synergid and daptomycin), no species of CoNS exhibited resistance to linezolid. Whereas, 1(6.25%) strain of *S.hominis-homin* and 1(100%) strain of *S.hominis-novo* showed resistance against daptomycin. For synergid, 3(2%) strains of *S.epidermidis* and 1(100%) strain of *S.capitis* were resistant.

Discussion

CoNS are increasingly being incriminated as a significant pathogen associated with healthcare infections and, therefore, there is a need for identification of CoNS up to species level as has been emphasised by many investigators.^{11,12} The species identification is important in monitoring the reservoir and distribution of CoNS involved in healthcare-associated infections and will help to understand the pathogenic potential of individual CoNS species.

In agreement with other studies, the current study showed that *S.epidermidis* was the most common among the CoNS species, causing sepsis in the NICU.^{13,14} In our study all CoNS isolates were from blood culture and among which *S.epidermidis* 144(75.8%) was found to be the most common species, followed by *S.haemolyticus* 21(11.1%) and *S.hominis-homin* 16(8.4%). Similar results were reported in a study from India¹⁵ in which the order of appearance of the three species was the same, whereas there was variation in their percentages i.e., *S.epidermidis* 234(40%), *S.haemolyticus* 84(14%) and *S.hominis* 65(11%). In another Indian study, *S.haemolyticus* 12(66%) was isolated in higher percentage compared to *S.epidermidis* 30(50%) from blood specimens.¹¹ A study in Brazil¹⁴ also found *S.epidermidis* to be the most common etiologic agent in 57% of the blood stream infection in NICU, followed by *S.warneri* and *S.capitis* (21%, each).

In our study, CoNS species showed maximum resistance to penicillin 189(99.5%) and ampicillin 188(99%). These results are in agreement with other studies which found that 94(94%) of the CoNS were resistant to penicillin¹⁵ and

98% to ampicillin.¹⁶ In another study from India, CoNS species showed 120(80%) resistance both to penicillin and ampicillin.¹¹

CoNS are gaining more importance due to methicillin resistance and as a consequence being resistant to all β -lactam antibiotics. Methicillin-resistant coagulase negative staphylococci (MRCoNS) have become predominant organism in hospitalised patients.¹⁷ Our study also showed similar results and majority of the isolates were MRCoNS (93.6%). Data from worldwide SENTRY antimicrobial surveillance programme reported that irrespective of geographical locations, about 70-75% of CoNS are resistant to methicillin.⁸ However, some studies from India and Brazil showed slightly lower rate of methicillin resistance among CoNS; 66%¹⁵ and 49%¹⁶ in India, and 69% in Brazil.¹⁸

The increasing trend in the prevalence of MRCoNS has also been reported by few investigators. A study from India¹⁶ reported an increase in MRCoNS during 2008-10; from 41.5% in 2008, 47% in 2009 to 57% in 2010. A similar trend in MRCoNS was reported from Brazil during 1990-2009. They found 51.6% MRCoNS during 1990-94, which increased to 63.6% during 1995-2002, 78.3% during 2003-06 and 87.5% in 2007-09.¹⁸ High rates of MRCoNS (87%) among neonatal isolates were also reported.¹⁹ Our results are quite close to the latter study¹⁹ as we found that 93.6% strains were MRCoNS, which is higher compared to the other studies.

In the present study, 95% of the isolates each of *S.epidermidis* and *S.haemolyticus* were resistant to oxacillin. These results are in agreement with the studies conducted in the Netherland²⁰ and Brazil.²¹ In the Dutch study, 97% of *S.epidermidis* isolates from an NICU during 1999-2001 were oxacillin-resistant. Whereas, the latter study from Brazil²¹ reported that 96% of *S.haemolyticus* strains were resistant to oxacillin and *S.haemolyticus* species was the second most prevalent MRCoNS, which we also found in our study. Another study from Brazil reported similar results, where 73.2% isolates of *S.epidermidis* and 85.7% isolates of *S.haemolyticus* were resistant to oxacillin.¹⁸

Resistance to erythromycin was also high in our study 148(78%). These results are quite in agreement with the SENTRY study⁸ which reported similar pattern of resistance for erythromycin from countries and regions like USA (81.3%), Canada (79%), Latin America (70.7%), Europe (72%) and Western Pacific (66%). However, a higher resistance to erythromycin (96%) was reported in a study from India for CoNS isolates.¹⁶

In our study, resistance to clindamycin was found to be 60%. A variable rate of resistance to clindamycin was reported in the SENTRY study for different locations: USA 50.9%, Canada

46.5%, Latin America 52.6%, Europe 48% and Western Pacific 36%.⁸ In contrast, quite high drug resistance to clindamycin (93%) was reported in a study from India.¹⁶

Like other studies,^{11,14,15} CoNS isolates in our study were also uniformly susceptible to vancomycin. However, CoNS isolates with decreased susceptibility to vancomycin have also been reported.^{8,22,23}

The in vitro activity of the new antimicrobial agents e.g., linezolid, daptomycin and synercid was compared with that of vancomycin. These agents demonstrated excellent in vitro activity against the isolates. All strains were 100% sensitive to linezolid, whereas low resistance was shown by the CoNS strains to daptomycin 3(1.6%) and synercid 4(2.2%). Similarly, in the SENTRY study 0.1% resistance was shown to linezolid.⁸

Conclusion

High rates of resistance were seen among CoNS isolates to commonly-used antibiotics, except to vancomycin and linezolid, which were 100% sensitive. The increasing rate of methicillin resistance and introduction of new antimicrobial agents increases the importance of local or national studies to be conducted periodically which can assist in planning to control the emergence and spread of antimicrobial resistance.

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