

Original Research Article

Molecular Study of *Staphylococcus* Species Isolated from Children with Sepsis in Pediatric Intensive Care: One Egyptian Center Study

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ABSTRACT

Staphylococcus species (*Staphylococcus* spp.) has emerged as a major cause of sepsis. Methicillin resistance is a pattern of resistance which hinders the management of such infection. The aims of the present study were to detect *Staphylococcus* spp. associated with sepsis in children admitted in PICU and the prevalence of *mecA* gene and to evaluate the phenotypic detection of methicillin resistance pattern by cefoxitin disc diffusion and oxacillin disc compared with molecular detection of *mecA*. Infants and children complaining of sepsis admitted to the pediatric intensive care unit (PICU) were recruited in the study. Blood cultures were performed and *Staphylococcus* species isolates were studied for detection of *mecA* gene by polymerase chain reaction (PCR) and by disc diffusion with cefoxitin and oxacillin discs. *Staphylococcus aureus* was isolated from 15.4% patients and CNS isolated from 88 patients (84.6%). *Staphylococcus coagulase* negative was the commonest isolates with *S.epidermidis* isolated from 28.8% followed by *S.sciuri* (17.3%). Among *Staphylococcus* sp. 54 species had *mecA* gene. The majority of isolated *S.aureus* had *mecA* gene (75%) compared to CNS (47.7%). The sensitivity of cefoxitin disc was 100% while that of oxacillin was 85% while the specificity of oxacillin disc was 86.2%. In conclusion, *Staphylococcus* species is a mundane pathogen isolated from pediatric intensive care unit. *Staphylococcus coagulase* negative species is the prevalent species with *Staphylococcus epidermidis* and *Staphylococcus sciuri* the most encountered species. Resistance to methicillin is a frequent finding with *mecA* gene presence. Cefoxitin disc diffusion method is a sensitive phenotypic method to detect methicillin resistance.

Keywords

Staphylococcus spp.,
PICU,
mecA

Introduction

Staphylococcus species (*Staphylococcus* spp.) are common pathogenic bacteria isolated in clinical laboratories worldwide. Though *Staphylococcus aureus* (*S. aureus*) is the common pathogenic species, non *aureus* species known as *Staphylococcus coagulase*

negative species (CNS) are now well known as pathogenic organisms, especially in immunocompromized patients in different geographical locations (Adeyemi et al., 2010, Reddy et al., 2010).

CNS is considered an opportunistic pathogen being isolated from hospital acquired infection (HAI) especially with the extended use of medical devices. The capacity of CNS to form biofilm within foreign prostheses or medical devices (Diaz et al., 2005). Higher percentages of isolated CNS previously reported are *Staphylococcus epidermidis* (76%) and *Staphylococcus haemolyticus* (32%) (Marino et al., 2001, Casey et al., 2007, Diaz et al., 2008). However, other CNS species can be associated with (HAI).

Among susceptible patients in hospital for CNS are neonates and children in pediatric intensive care units (PICU). CNS is associated with high morbidity and mortality rates and prolonged hospital stay (Geme and Harris 1991, Stoll et al., 1996, Mittendorf et al., 1999 - Stoll et al., 2002, Isaacs, 2003, Healy et al., 2004, Liljedahl et al., 2004, Stoll et al., 2004).

The laboratory diagnosis of invasive infections caused by CNS carries a challenge for clinical microbiologists as these organisms are considered normal human flora, therefore microbiological culture isolation may indicate culture contamination. Several laboratory definitions of CNS sepsis exist. Some investigators have defined CNS infection as either two positive blood cultures drawn within two days of each other or one positive blood culture, and observed elevated C-reactive protein within two days of the CNS positive culture (Stoll et al., 2002).

Staphylococcus spp. is known to have resistance to multiple antibiotics especially to beta lactams antibiotics and especially for methicillin (Gould et al., 2012). The accurate reporting of susceptibility pattern of isolated *Staphylococcus* sp. is of utmost importance for effective clinical

management of those patients with choosing appropriate modalities of therapeutic regimen. The mechanism of Methicillin resistance depends mainly on the production of altered penicillin-binding protein (PBP), 2a coded by the *mecA* gene complex (Dumitrescu et al., 2010). This altered PBP 2a in the bacterial cell walls is the cause of diminished susceptibility to methicillin. In heterogeneous pattern of resistance all bacterial cells in an intrinsically resistant organism carry the *mecA* gene; nevertheless, it is only expressed by a small number of bacterial cells. This phenomenon explains why routine tests may fail to detect methicillin resistance in *Staphylococcus* spp. The cefoxitin disc diffusion method is considered a better indicator than oxacillin for the detection of heterogeneous methicillin resistance and is recommended by the CLSI (Swenson and Tenover, 2005, CLIS, 2008).

Molecular detection of the *mecA* gene by polymerase chain reaction (PCR) is the most accurate method for detection of clinical *Staphylococcus* spp. methicillin resistant isolates (Majouri et al., 2007, Akpaka et al., 2008, Mohanasoundaram and Lalitha, 2008). Laboratories use a variety of phenotypic tests for the detection of methicillin resistance in staphylococci. These phenotypic screening methods include disk diffusion tests with either cefoxitin (Cefox) or moxalactam (Moxa) on Mueller Hinton (MH) agar incubated at 37°C, oxacillin on MH agar incubated at 30°C (Oxa30) and oxacillin on salt MH agar incubated at 37°C (Oxa37). It is also possible to detect PBP 2a in isolates using a commercial agglutination test (Seydi et al., 2004, Affolabi et al., 2012).

The aims of the present study were to detect *Staphylococcus* spp. associated with sepsis in children admitted in PICU and the

prevalence of *mecA* gene and to assist the phenotypic detection of methicillin resistance pattern by cefoxitin disc diffusion and oxacillin disc compared with molecular detection of *mecA*.

Material and Method

Infants and children between 6 months and 15 years of age complaining of sepsis admitted to the PICU of Mansoura University Children's Hospital during a period of one year from January 2014 till January 2015 were recruited in the study. This study was approved by the ethical committee of Mansoura Faculty of Medicine and the parents of each child signed a written informed consent.

Clinical sepsis was “defined as the presence of three or more of the following categories of clinical signs derived from a validated sepsis score: (a) temperature instability (hypothermia, hyperthermia); (b) respiratory (grunting, intercostal retractions, apnea, tachypnea, cyanosis); (c) cardiovascular (bradycardia, tachycardia, poor perfusion, hypotension); (d) neurologic (hypotonia, lethargy, seizures); (e) gastro intestinal (feeding intolerance, abdominal distension) (Tollner, 1982)”. A full laboratory sepsis screen which included cerebro-spinal fluid analysis and cultures from blood, urine, endotracheal aspirate (infants on mechanical ventilation) and culture of indwelling central lines was performed.

Microbiological methods

Blood samples of patients with suspected bacteraemia were analyzed using the Bactec 9050 automated blood culture system (Becton Dickinson Diagnostic systems, Maryland, USA). Positive blood cultures were isolated on blood agar and mannitol salt agar plates (Oxoid, Basingstoke, UK)

and plates were incubated at 35°C for 24 hours. Organisms were detected based on Gram staining, standard biochemical tests and colony morphology. Identification to the species level was done using the MicroScan® Walk Away diagnostic microbiology system (Siemens HealthCare Diagnostics, formerly Dade Behring, USA).

Antibiotic susceptibility

Antibiotic susceptibilities of the isolated strains were determined by the disk diffusion method on Mueller-Hinton agar plates according to the regulations of the Clinical and laboratory Standards Institute (CLS, 2007). The used discs were oxacillin (1µg) disc, cefoxitin (30 µg), Flouroquinolone (5 µg), ceftazidime(30 µg), ceftriaxone (30 µg), cefoperazone (75 µg) , ampicillin (10 µg) , piperacillin (100 µg), vancomycin (5 µg), cefotaxime (30 µg).

PCR Detection for *mecA*

DNA extraction

Staphylococcal DNA was isolated by classical chloroform, phenol extraction method as previously described (Maniatis, et al., 1982).

The primers used for detection of *mecA* gene were (*mecA* F: 5' GTAGAAAT GACTGAA CGTCCGATGA 3' and *mecA* R: 5' CCAA TTCCACATTGTTTCGGTCTAA 3'). The reaction condition was similar to that previously reported (Geha, et al., 1994).

The PCR reagent mixture consisted of 200uM deoxynucleoside triphosphates, 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 uM MgCl₂, 0.5% Tween, 10% glycerol, 50 pmol of *mecA* primers, 0.5% isopsoralen compound 10 and 1.25 U of Ampli Taq DNA polymerase (Perkin-Elmer Cetus,

Norwalk, Conn.) Then add DNA extract solution 2 microns and water to be added to the PCR mixture for a 50 microns reaction mixture.

A Biometra DNA thermocycler was programmed with the initial denaturation for 4 min at 94°C; then 30 cycles with a 45seconds denaturation step at 94°C, a 45 second annealing step at 56°C and a 30 seconds extension step at 72°C and 2 min and a holding step at 4°C until the sample was analyzed. The PCR products were electrophoresed, stained with 10µM ethidium bromide and visualized by using UV transillumination. Amplification with these primers gave rise to a 310-bp *mecA*-specific product.

Statistical analysis

The results were analyzed by the use of SPSS (Statistical package for the Social Sciences) version 16. Sensitivity, specificity, positive values and negative predictive values were calculated for cefoxitin disc and oxacillin disc compared to detection of the *mecA* gene by PCR by using the following equations:

Sensitivity divides TP by (TP+FN) x100.

Specificity, divides TN by (FP+TN) x100

Positive predictive value, divides TP by (TP+FP) x100)

Negative predictive value divides TN by (TN+FN) x100

TP=true positive, FN=false negative, FP false positive, TN=true negative.

Results and Discussion

Among affected infants and children admitted to PIUC during the period from January 2014 to January 2015 gram positive sepsis was identified in 120 blood samples.

From these isolates, 104 were identified as *Staphylococcus species*.

Table 1 summarized *Staphylococcus sp.* Isolated from our patients. *Staphylococcus aureus* was isolated from 15.4% patients and CNS isolated from 88 patients (84.6%). *Staphylococcus coagulase negative* was the commonest isolates with *S.epidermidis* isolated from 28.8% followed by *S.sciuri* (17.3%), table 1.

Figure 1 & table 2 represented the resistance pattern among isolated *Staphylococci* species. The major resistance pattern was toward ampicillin and ceftriaxone (55.8%). Resistance for oxacillin disc was 25% and for cefoxitin was 32.7%. Antibiotics resistance was common in both *S.aureus* and CNS. The highest resistance was for ampicillin, and cefotaxime for *S.aureus* (75 % for each) and for CNS (63.6 % & 50 %, respectively). Methicillin resistance of *S.aureus* was mainly toward oxacillin (75%) while for CNS it was mainly toward cefoxitin (100%).

Among *Staphylococcus sp.* 54 species had *mecA* gene. The majority of isolated *S.aureus* had *mecA* gene (75%) compared to CNS (47.7%), table3

The sensitivity and specificity discs methods compared to *mecA* detection by PCR for detection of methicillin resistant staphylococcus spp. are shown in table 4. Oxacillin disk diffusion tests had the lower sensitivity (85%) than that of cefoxitin disc (100%) though had higher specificity (86.6%).

CNS is normal flora in human and turn to be pathogenic bacteria in immunocompromised patients (Raad et al., 1998). CNS is identified as pathogenic etiology in up to 80% of sepsis in neonates. The most

frequent isolated CNS species is *Staphylococcus epidermidis* followed by *Staphylococcus haemolyticus* (Hira et al., 2007, Gheibi et al., 2008, Dimitriou et al., 2011).

In the present study CNS was isolated from 88 patients (84.6%) admitted to PICU with the commonest isolates *S.epidermidis* 28.8% followed by *S.sciuri* (17.3%).

Staphylococcus epidermidis represented main bacterium found on human skin. Infection in intensive care units by this bacterium usually occurs by contaminating of the medical device. The contamination of intravenous devices like intravenous line is associated with the production of a virulence factor known as the ability of organism to form biofilm (Costerton et al., 1995).

Staphylococcus sciuri, is another common CNS isolated from our patients, is well known pathogens found to be associated with numerous infections and with septic shock (Chen et al., 2007).

Staphylococcus aureus was isolated from 15.4% patients. Previous reports in adults intensive care units the isolation rates for *Staphylococcus aureus* was reported in 35 (25.7 %) (Lim et al., 2014), while in children it was *Staphylococcus epidermidis* (55.4%), *Staphylococcus aureus* (9.5%) (Babay et al., 2005). These results highlight the importance of CNS as a pathogenic bacterium in PICU.

The present study highlights the presence of multi-drug resistance among the isolated staphylococci species. Antibiotics resistance was common in both *S.aureus* and CNS. The highest resistance was for ampicillin, and cefotaxime for *S.aureus* (75% for each)

and for CNS (63.6% & 50% respectively). Methicillin resistance of *S.aureus* was mainly toward oxacillin (75%) while for CNS it was mainly toward cefoxitin (100%).

These findings are concurrent with previous data where isolated staphylococci species from neonatal intensive care units may show resistance to methicillin up to 100% with connection to multiple resistance to other antibiotics (Qu et al., 2010, Abd El Hafez et al., 2011), especially to betalactams antibiotics. (Brzychczy-Wloch et al., 2013). Lower rates of oxacillin-resistant *Staphylococcus* species were reported in developed countries like United Kingdom 15.1% (Denniston et al., 2006) and in USA 16.3% and in New Zeland 12.7% (Pereira et al., 2014). However the rate increased in other study from USA to reach 47.4% (Dolapo et al., 2014).

These results indicate that Methicillin-resistant *Staphylococcus* spp. are common pathogen in PICU and can cause catastrophic outcome in those affected patients due to associated risk factors like use of invasive procedures (Kuint et al., 2007). Thus the rapid detection of these organisms is an important issue to perform adequate management of health care associated infections (Sloos et al., 2000).

Investigation of the *mecA* gene in CNS using the PCR technique is nowadays regarded as the gold standard with a view to determining methicillin resistance (Pereira et al., 2010). The rate of methicillin resistant *Staphylococcus* species detected by cefoxitin disc was higher for both *S.aureus* (75%) and CNS (100%) than the proportion (60-70%) reported in some hospitals in developed countries in Europe (Stefani and Varaldo, 2003).

Table.1 *Staphylococcus sp.* Isolated from patients

<i>Staphylococcus sp.</i>	No. (%)
<i>S. aureus</i>	16 (15.4%)
<u>CNS</u>	<u>88(84.6%)</u>
<i>S. epidermidis</i>	30(28.8%)
<i>S. sciuri</i>	18(17.3%)
<i>S. hominis</i>	14(9.6%)
<i>S. saprophyticus</i>	9(3.8%)
<i>S. cohnii</i>	5(3.8%)
<i>S. xylosus</i>	5(3.8%)
<i>S. intermedius</i>	3(1.9%)
<i>S. hyicus</i>	3(1.9%)
<i>S. haemolyticus</i>	1(1.9%)
Total	104(100%)

Table.2 Resistance pattern among isolated *Staphylococcus* species

	<i>S. aureus</i>		CNS		P
	No.	%	No.	%	
Oxacillin	12	75%	50	56.8 %	0.2
Cefoxitin	6	37.5%	88	100%	0.4
Flouroquinolone	0	0%	28	31.8%	0.03
ceftazidime	0	0%	36	40.9%	0.007
ceftriaxone	12	75%	50	56.8 %	0.2
cefoperazone	6	37.5%	28	31.8%	0.7
ampicillin	12	75 %	56	63.6%	0.2
cefotaxime	12	75%	44	50%	0.02
Piperacillin	0	0%	34	38.6%	0.01
vancomycin	0	0%	32	36.4%	0.02
Total	16	100%	88	100%	

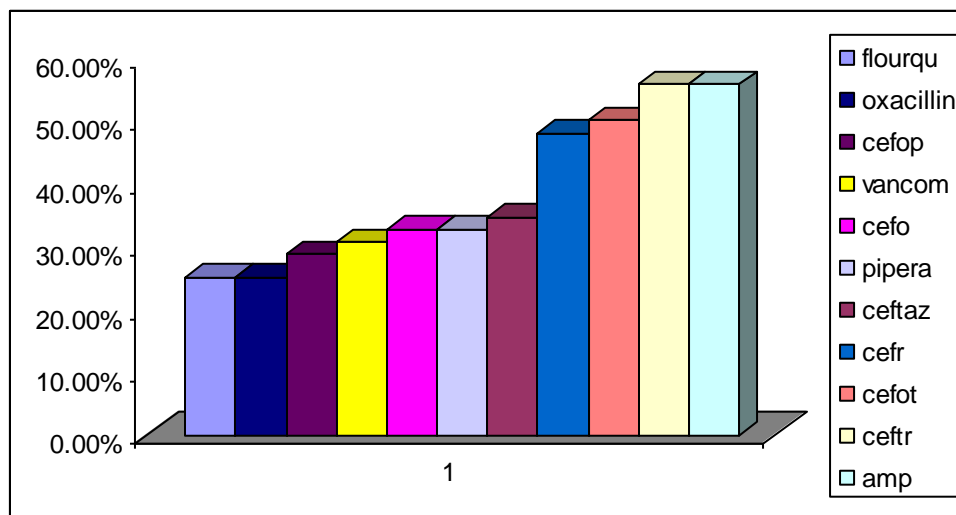
Table.3 *mec A* among isolated *Staphylococcus sp.*

<i>mecA</i> gene	<i>S.aureus</i> No. %	CNS NO. %
Positive	12 75%	42 47.7%
Negative	4 25%	46 52.3%
Total	16 100%	88 100%

P=.04

Table.4 Detection of methicillin resistance among isolated Staphylococcus species by phenotypic and genotypic methods

	sensitivity	specificity	Positive predictive value	Negative Predictive value
cefoxitine	100 %	55.6 %	57.5%	100%
oxacillin	85%	86.2%	87.1%	100%



cefoxitin (cefo), Flouroquinolone (flourq), ceftazidime(ceftaz), ceftriaxone (cefr), cefoperazone (cefop) , ampicillin (amp), piperacillin (pipera), vancomycin (vancom), cefotaxime (cefo)

The presence of emergence of methicillin resistant Staphylococci species in this study reflects the ability of these strains to spread within and between hospitals (Widerström et al., 2006, Monsen et al., 2000) through hands of health care staff and equipments. Special therapeutic modalities should be thought for those patients to treat the infections and to prevent further uncontrolled spread of these species. Mupirocin's appears as a good therapeutic modality (Perl et al., 2002).

The presence of *mecA* gene was confirmed with PCR method in staphylococcus isolates in the present study. The majority of isolated *S.aureus* had *mecA* gene (75%) compared to CNS (47.7%). In previous study the rate of detection of *mecA* gene in CNS was up to 100% (Brzychczy-Wloch et al., 2013).

Methicillin resistance in *Staphylococci* sp. is acquired from the presence of staphylococcal cassette chromosome *mec* (SCC*mec*) which is known as mobile genetic element carrying *mecA*. There is eight SCC*mec* types that differs in the size and in the allotypic combination of the *mec* (A, B, C) (Zhang et al., 2009).

The limitations of the present study were the limited number of the used primers for detection of *mec* genes types. This could explain the lower rate of *mecA* detection among Staphylococcus sp. identified by disc susceptibility as methicillin resistant.

The present study demonstrated that oxacillin disk diffusion tests had the lower sensitivity (85%) than that of cefoxitin disc (100%) though had higher specificity

(86.2%). These findings are online with other studies (Datta et al., 2011, Olowe et al., 2013, Affolabi et al., 2014). Though this test is still in use in many of our microbiological laboratories, we would recommend using cefoxitin as screening test according to our study.

In conclusion, staphylococcus species is a mundane pathogen isolated from pediatric intensive care unit. Staphylococcus coagulase negative species is the prevalent species with *Staphylococcus epidermidis* and *Staphylococcus sciuri* the most encountered species. Resistance to methicillin is a frequent finding with *mecA* gene presence. Cefoxitin disc diffusion method is a sensitive phenotypic method to detect methicillin resistance.

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