

Phenotypic and Genotypic Investigation of Methicillin Resistant Staphylococci Species Isolated from Children with Sepsis in Egypt

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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 isolate Staphylococci spp. from children with clinical signs and symptoms of sepsis at Mansoura University Children Hospital (MUCH), Egypt. Then to detect methicillin resistance Staphylococci (MRS) using primary screening test followed by molecular typing of the methicillin resistant Staphylococci isolates by multiplex PCR. MRS sepsis was diagnosed in a total 100 patient from February 2015, till February, 2016. 18 (18%) of isolates were identified as *S. aureus* and 82 (82%) of *Staphylococci* spp. were identified as coagulase negative Staphylococci (CoNS). *S. hominis* subsp. *hominis* was the commonest species of CoNS (32/82, 39%), followed by *S. sciuri* (9/82, 11%). the rate of Oxacillin resistance among all isolated Staphylococci strains was (100%) in both *S. aureus* and CoNS isolates.

Among the 50 selected resistance species for multiplex PCR there 27 (54%) species had a single gene *SCCmec* type I or *SCCmec* type (II or III). While 13 (26%) isolates had both two genes (*SCCmec* type I + II or III) and 10 (2%) had no *SCCmec* genes. The spectrum of children with sepsis at Mansoura University Children Hospital (MUCH), confirmed the importance of pathogens such as *Staphylococcus* spp. Similar to other studies from the developing countries, CoNS species were the most common isolated pathogens in children with sepsis.

Keywords: Coagulase negative Staphylococci, Methicillin resistant Staphylococci, Mansoura University Children Hospital, Staphylococcal Cassette Chromosome *mec*

Introduction

In the last decade, *Staphylococcus* has emerged as predominant microorganism in nosocomial infections (Kim, 2009). The main staphylococcal infections reported include folliculitis, furuncles, serious toxic shock syndrome and sepsis (Casey et al., 2007 and Haamann et al., 2011).

Staphylococci are the most abundant isolated bacteria from blood (Yalaz, 2006 and Nicoleta et al., 2016). Since septicemia with *Staphylococcus aureus* is associated with a high mortality and an increased length of stay in hospital, timely detection and identification of *S. aureus* or coagulase-negative Staphylococci

(CoNS) including methicillin resistance from the patient's blood has great therapeutic, economic and prognostic significance (Gröbner & Kempf, 2007).

Methicillin resistance occurs in *Staphylococcus* species due to the expression of an altered penicillin binding protein termed PBP2a that is encoded by the *mecA* gene (Katayama et al., 2000). The *mecA* gene is located on a mobile staphylococcal cassette chromosome *mec* (*SCCmec*) element. This element exists in methicillin resistant *Staphylococcus aureus* (MRSA); as well as, methicillin-resistant Coagulase negative Staphylococci (MRCoNS)

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isolates. To date, 11 types of SCC mec have been identified in *S. aureus* and in *Staphylococcus epidermidis* (Helen et al., 2008 and International Working Group on the Classification of Staphylococcal Cassette Chromosome (SCC) Elements (IWG-SCC), 2009).

Identification of methicillin resistance is performed by phenotypic and genotypic methods (Martins, 2007 and Nicoleta et al., 2016). The methods that stand out among the most utilized or best capable in the identification of MRSA and MRCoNS are: Agar dilution method, disk diffusion, screening on agar with methicillin, automated methods (Microscan, Vitek), latex agglutination and the molecular method using PCR (Ferreira et al., 2003; Antunes et al., 2007; Anand et al., 2009; CLSI, 2010 and Shariati et al., 2010).

Accordingly, the objective of this study was to isolate Staphylococci spp. from children with clinical signs and symptoms of sepsis at Mansoura University Children Hospital (MUCH), Egypt, then to detect methicillin resistance Staphylococci (MRS) using primary screening test followed by molecular typing of the methicillin resistant Staphylococci isolates by multiplex polymerase chain reaction (PCR).

Materials and Methods

Study population

A total of 100 patients during one year of study period from February 2015, till February, 2016, between the ages of one month and 16 years having clinical features suggestive of sepsis (fever, shortness in breath, weakness, drowsiness, irritability, etc.) were collected from different departments of Mansoura University Children Hospital (MUCH), Egypt.

Sample collection

Two milliliter (mL) of blood samples from early age children and 5mL of blood samples from late age children were drawn for blood culture at the completion of the procedure. All samples were collected in sterile disposable containers, (taken to the laboratory without delay) and then subjected to the bacteriological assay within two hours.

Isolation and purification of bacterial isolates

Sterile dry swabs were used for streaking of blood samples onto sterile dishes containing nutrient agar media (Oxoid). Inoculated streaked media were incubated at 37°C for 48 h. Single colonies were picked and streaked on agar

surface of different media such as nutrient agar, MacConkey agar and blood agar.

Identification of staphylococcal bacterial isolates

Isolates obtained were identified according to Bergey's manual of Bacteriology (Holt et al., 1994), using; Gram staining, colony characteristics, and biochemical properties including catalase, coagulase (free and bound) and hemolytic activity on blood agar plates.

Identification of clinical isolates to species level

Identification of some Staphylococci to species level was confirmed by using the MicroScan WalkAway-96 SI System (Siemens Healthcare Diagnostics, USA) at Mansoura University Children Hospital (MUCH) by using the MicroScan Dried Gram Positive ID Type 2 (Pos ID Type 2) panels which were designed for *in-vitro* diagnostic use in determining identification to the species level of aerobic and facultative Gram positive cocci.

Antibiotic susceptibility

Susceptibility testing for coagulase-negative Staphylococci (CoNS) was performed on the MicroScan Walkaway system (Siemens Healthcare Diagnostics, USA) using Positive MIC Panel Type 2, with breakpoint ranges from resistant, susceptible and intermediate included the following antibiotics, Amoxicillin-clavulanate (Aug), Ampicillin-sulbactam (A/S), Azithromycin (Azi), Cefazolin (Cfz), Cefepime (Cpe), Cefotaxime (Cft), Ceftriaxone (Cax), Cephalothin (Cf), Chloramphenicol (C), Ciprofloxacin (Cp), Clindamycin (CD), Erythromycin (E), Gatifloacin (GAT), Gentamicin (Gm), Imipenem (IPM), Levofloxacin (LVX), linezolid (LZD), Moxifloxacin (MXF), Ofloxacin (OFI), Oxacillin (Ox), Rifampin (Rif), Synercid (SYN), Tetracycline (TE), Trimethoprim/sulfamethoprim (SXT), Vancomycin (VA), Penicillin (PEN) and Ampicillin (AM).

Susceptibility testing for *S. aureus* was determined by disk diffusion method. The used discs were Oxacillin (OX) (1 µg) disc, Clindamycin (DA) (2 µg), Vancomycin (VA) (30 µg), Rifampin (RD) (5 µg), Trimeth/sulfa (SXT) (25 µg), linezolid (LZD) (30 µg), Imipenem (IPM) (10 µg). Categorical results and the susceptibility profiles of each antimicrobial agent tested were based on the Clinical Laboratory Standards Institute (CLSI) interpretative criteria (Patel, 2015).

Molecular methods

DNA extraction: Genomic DNA of (50) selected Staphylococci species was isolated using the Thermo Scientific GeneJET Genomic DNA Purification Kit (supplied by Thermo Scientific) according to the procedure of the kits.

Polymerase chain reaction assay for typing of SCCmec

Two loci (A and C) were chosen for multiplex polymerase chain reaction (PCR) (Oliveira & de Lencastre, 2002) to type the staphylococcal cassette chromosome *mec* element (SCC*mec*) in methicillin resistant Staphylococci (MRS). The 2 loci are shown in Table A.

TABLE A. Primers used in polymerase chain reaction to type the staphylococcal cassette chromosome *mec* (SCC*mec*) element in methicillin resistant Staphylococci.

Locus	Primer	Oligonucleotide sequence (5 – 3)	Amplicon size (bp)	Specificity (SCC <i>mec</i> type)	References
A	CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	495	I	(Oliveira and de Lencastre,2002)
	CIF2 R2	ATTACCACAAGGACTACCAGC			
C	MECI P2	ATCAAGACTTGCAATTCAGGC	209	II , III	(Oliveira and de Lencastre,2002)
	MECI P3	GCGGTTTCAATTCACCTTGTC			

Multiplex PCR was performed in 25 µl final reaction mixture 5 µl of prepared DNA template having a final concentration 10 µg/ml, 2.5 µl of 10x Taq DNA polymerase buffer with KCl, 2.5 µl of MgCl₂ (25 mM), 0.2 µl of Taq DNA polymerase (5 U/µl), 1 µl of 5 mM dNTPs, 13.4 µl of nuclease free distilled water and 0.2 µl for first primer and 0.2 µl for second primer.

TABLE 1. The demographic characteristics of the study patients.

Age	Sex				Total	
	Female		Male		No.	%
	No.	%	No.	%		
<1 year	24	24	26	26	50	50
1- 5 year	16	16	12	12	28	28
6-10 year	1	1	7	7	8	8
11-16 year	8	8	6	6	14	14
Total	49	49	51	51	100	100

82 (82%) of *Staphylococci* species were identified as CoNS and 18 (18%) were identified as *S. aureus*. Species level of 82 CoNS isolates were identified by MicroScan Dried Gram Positive ID Type 2 (Pos ID Type 2) (panels are shown in Table 2) where *S. hominis* subsp. *hominis* was the commonest species of CoNS (32/82, 39%), followed by *S. sciuri* (9/82,

Results

A total of 100 patients were enrolled in the study, 49% of whom were females and 51% were males (Table 1). The age of patients enrolled in the study ranges between one month to 16 years. The highest ratio was in the patient of the age less than one year (50%), while the lowest was in patients of age interval from six to ten years (8%) as showed in Table 1.

11%). *S. epidermidis*, *S. haemolyticus*, and *S. simulans* each of them represented (8/82, 9.8%). *S. auricularis* represented (5/82, 6.1%), each of *S. saprophyticus* & *S. cohnii- cohnii* was (3/82, 3.7%), While *S. xylosus* represented by (2/82, 2.4%). The lowest isolates ratios were in *S. intermedius*, *S. capitis-capitis*, *S. caprae* and *S. hominin-novo* represented by (1/82, 1.2%).

TABLE 2. Percentage of *Staphylococci* species level isolated from patients.

Coagulase negative <i>Staphylococci</i> (CoNS)	Total no. of isolated CoNS	Percent %	Coagulase positive <i>Staphylococci</i> <i>S. aureus</i>	Total no. of isolated <i>S. aureus</i>	Percent %
<i>S. hominis subsp. hominis</i>	32	39 %	<i>S. aureus</i> 1	18	100 %
<i>S. sciuri</i>	9	11 %	<i>S. aureus</i> 2		
<i>S. haemolyticus</i>	8	9.8 %	<i>S. aureus</i> 3		
<i>S. epidermidis</i>	8	9.8 %	<i>S. aureus</i> 4		
<i>S. simulans</i>	8	9.8 %	<i>S. aureus</i> 5		
<i>S. auricularis</i>	5	6.1 %	<i>S. aureus</i> 6		
<i>S. saprophyticus</i>	3	3.7 %	<i>S. aureus</i> 7		
<i>S. cohnii-cohnii</i>	3	3.7 %	<i>S. aureus</i> 8		
<i>S. xylosum</i>	2	2.4 %	<i>S. aureus</i> 9		
<i>S. intermedius</i>	1	1.2 %	<i>S. aureus</i> 10		
<i>S. capitis-capitis</i>	1	1.2 %	<i>S. aureus</i> 11		
<i>S. caprae</i>	1	1.2 %	<i>S. aureus</i> 13		
<i>S. hominin-novo</i>			<i>S. aureus</i> 14		
			<i>S. aureus</i> 15		
	1	1.2 %	<i>S. aureus</i> 16		
			<i>S. aureus</i> 17		
			<i>S. aureus</i> 18		
Total of CoNS	82	100 %	Total of <i>S. aureus</i>		18

The susceptibility testing of CoNS bacteria are illustrated in Table 3. CoNS isolates showed complete resistance Amoxicillin-clavulanate, Ampicillin-sulbactam, Cefazolin, Cefepime, Cefotaxime, Ceftriaxone, Cephalothin and Oxacillin (%R=100) and showed high resistance to Imipenem (%R=98.8), Erythromycin (%R=82.9) and Azithromycin (%R=81.7). Intermediate effect was observed with Levofloxacin, Ciprofloxacin, Trimeth/sulfa and Tetracycline. Best sensitivity was also observed to Vancomycin (%R= 12.2).

The susceptibility testing of *S. aureus* bacteria are illustrated in Table 4. All *S. aureus* were completely resistant to Oxacillin and showed high resistant to Clindamycin (% R=83.3) and Vancomycin (% R=72.2). Intermediate effect (% R= 44.4 – 55.6) was observed with Imipenem and Linezolid.

Among the 50 selected resistant species for multiplex PCR there 27(54%) species had a single gene including SCCmec type I or SCCmec type (II or III). While 13 (26%) isolates had both two genes (SCCmec type I+ II or III) and 10 (2%) had no SCCmec genes and as shown in Table 5 and Fig. 1.

Mec element locus (A) specific to SCCmec type I gene among tested methicillin resistant *Staphylococci* species results showed in Table 6. MRSA samples were positive in 13(72.2%) and 20(62.5%) in MRCoNS isolates with total of 33(66%) MRS samples, while negative results were detected in 5(27.8%) and 12 (37.5%) with MRSA and MRCoNS samples, respectively. The amplified region of the Mec element locus (A) was detected on agarose gel electrophoresis at 495 bp.

TABLE 3. Antimicrobial susceptibility testing of CoNS (MRCoNS) by MicroScan Walkaway system.

Type of antibiotic	Susceptible		Resistant		Intermediate	
	No	%	No	%	No	%
*Amox/K Clav	0	0	82	100	0	0
**Amp/sulbactam	0	0	82	100	0	0
Azithromycin	12	14.6	67	81.7	3	3.7
Cefazolin	0	0	82	100	0	0
Cefepime	0	0	82	100	0	0
Cefotaxime	0	0	82	100	0	0
Ceftriaxone	0	0	82	100	0	0
Cephalothin	0	0	82	100	0	0
Chloramphenicol	60	73.2	17	20.7	5	6.1
Ciprofloxacin	35	42.7	47	57.3	0	0
Clindamycin	41	50	33	40.2	8	9.8
Erythromythin	9	11	68	82.9	5	6.1
Gatifloacin	42	51.2	40	48.8	0	0
Gentamicin	29	35.4	49	59.8	4	4.9
Imipenem	1	1.2	81	98.8	0	0
Levofloxacin	37	45.1	45	54.9	0	0
Linezolid	64	78	18	22	0	0
Moxifloxacin	48	58.5	30	36.6	4	4.9
Ofloxacin	24	29.3	55	67.1	3	3.7
Oxcillin	0	0	82	100	0	0
Rifampicin	55	67.1	23	28	4	4.9
Syercid	55	67.1	20	24.4	7	8.5
Tetracycline	30	36.6	48	58.5	4	4.9
Trimeth/sulfa	35	42.7	47	57.3	0	0
Vancomycin	71	86.6	10	12.2	1	1.271

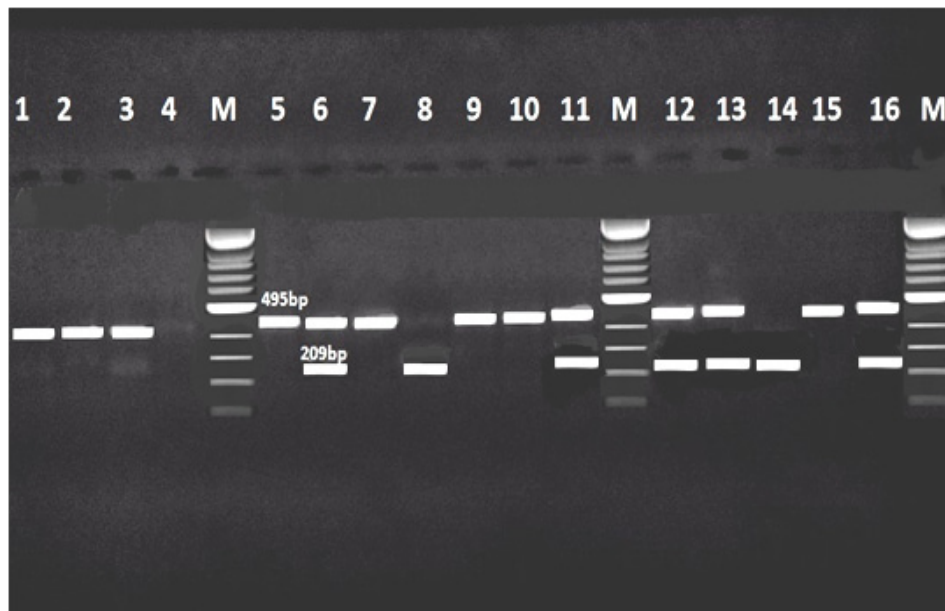
*Amox/K Clav: Amoxicillin-clavulanate ** Amp/sulbactam: Ampicillin-sulbactam.

TABLE 4. Antibiotic susceptibility testing of *S. aureus* (MRSA) by disk diffusion method.

Type of antibiotics	Sensitive		Resistant	
	No.	%	No.	%
Oxcillin	0	0	18	100
Clindamycin	3	16.7	15	83.3
Vancomycin	5	27.7	13	72.2
Rifampicin	6	33.3	12	66.7
Trimeth/sulfa	6	33.3	12	66.7
Linezolid	8	44.4	10	55.6
Imipenem	10	55.6	8	44.4

TABLE 5. Distribution of the typable species according to number of SCCmec types detected in the same isolate.

Category	Isolate no.	No. of isolates (%)
One SCCmec type {SCCmec type I or SCCmec type (II or III)}	89, 90, 93, 95, 96, 98, 99, 33, 34, 38, 39, 40, 41, 43, 45, 46, 47, 48, 50, 53, 54, 56, 57, 58, 59, 60, 62.	27 (54%)
Two SCCmec type {both genes SCCmec type I + SCCmec type (II or III)}	2, 87, 88, 91, 94, 97, 32, 35, 36, 37, 42, 52, 63.	13 (26%)
-ve SCCmec	1, 3, 4, 92, 100, 44, 49, 51, 55, 61.	10 (2%)
Total		50

**Fig. 1. Agarose gel electrophoresis of amplicons produced from Multiplex PCR for SCCmec typing among Staphylococci isolates.**

- Lane M was 100bp DNA Ladder.
- Lane (1:4): amplicons from Staphylococci isolates no. 47, 46, 45 and 44, respectively.
Where,
Isolates No.47, 46 and 45 harbouring one SCCmec types (I) only
Isolates No. 44 was negative (-ve SCCmec)
- Lane (5: 11): amplicons from Staphylococci isolates no.43, 42, 41, 40, 39, 38,37 respectively.
Where,
Isolates No. 43, 41, 39 and 38 harbouring SCCmec types (I) only
Isolates No. 41 and 37 harbouring SCCmec types (I + II or III)
Isolates No. 40 harbouring SCCmec types (III) only
- Lane (12:16): amplicons from Staphylococci isolates no. 36, 35, 34, 33, and 32, respectively.
Where,
Isolates No. 36, 35and 32 harbouring SCCmec types (I + II or III)
Isolates No. 34 harbouring SCCmec types (II or III)
Isolates No. 33 harbouring SCCmec types (I) only

TABLE 6. SCCmec type I gene PCR results among tested methicillin resistant Staphylococci species.

		*MRSA (n=18)		**MRCONS (n=32)		Total (n=50)	
		No.	%	No.	%	No.	%
***SCCmec type I Result	Positive	13	72.2	20	62.5	33	66
	Negative	5	27.8	12	37.5	17	34

*MRSA: Methicillin resistant *Staphylococcus aureus*.

**MRCONS: Methicillin resistant- Coagulase negative Staphylococci.

*** SCCmec: Staphylococcal Cassette Chromosome *mec*.

Mec element locus (C) which present in SCCmec type II and type III genes results among tested methicillin resistant Staphylococci species showed in Table 7. MRSA samples were positive in 6 (33.3%) and 14 (43.8%) in MRCONS isolates

with total of 20 (40%) MRS samples, while negative results were detected in 12 (66.7%) and 18 (56.2%) with MRSA and MRCONS samples, respectively. The amplified region of the Mec element locus (C) was detected on agarose gel electrophoresis at 209 bp.

TABLE 7. SCCmec type II or III gene PCR results among tested methicillin resistant Staphylococci species.

		*MRSA (n=18)		**MRCONS (n=32)		Total (n=50)	
		No.	%	No.	%	No.	%
***SCCmec type II or III Result	Positive	6	33.3	14	43.8	20	40
	Negative	12	66.7	18	56.2	30	60

*MRSA: Methicillin resistant *Staphylococcus aureus*.

**MR-CONS: Methicillin resistant- Coagulase negative Staphylococci.

*** SCCmec: Staphylococcal Cassette Chromosome *mec*.

Discussion

Staphylococcus is a pathogenic bacterium including more than 60 species and subspecies and causes many human and animal infections. In spite of being commensal on host skin, they become pathogens when gaining the entry into the host tissue or in cases in which the microbial community is disturbed or in immune-compromised individuals (Kloos & Bannerman, 1994).

In this study, the *Staphylococcus* bacterial sepsis was suspected more in male children (51%) than in female children (49%). Our result is in accordance with Nimri et al. (2001), Karki et al. (2010) and Ansari et al. (2014), who have also reported higher bacterial growth in male patients than female patients.

Coagulase negative Staphylococci (CoNS) were found to be the most common etiology of septicemia from 100 Staphylococci blood cultures isolates, 82 (82%) were coagulase negative

Staphylococci (CoNS). Our result is in agreement with other studies that reported CoNS is identified as pathogenic etiology in up to 80% of sepsis in neonates (Hira et al., 2007; Ghelbi et al., 2008 and Dimitriou et al., 2011).

CoNS were at the forefront of causative agents of children sepsis. For this reason, identification of the isolated offending species was of crucial importance to establish epidemiological trends, and to permit a more precise determination of the host-pathogen relationships of Staphylococci (Kleeman et al., 1993 and Gribaldo et al., 1997), that will help in the development of preventive and control measures, and in the initiation of appropriate antibiotic therapy so that a good clinical outcome can be guaranteed.

In our study, *Staphylococcus aureus* was isolated from 18% 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 (9.5%) (Babay et al., 2005).

Different methods have been described for identification of *Staphylococcus* spp.; in our study we used MicroScan® Walk Away system for identification of coagulase negative Staphylococci (CoNS) to species level.

Among CoNS isolates in our study *S. hominis* subsp. *hominis*, *S. sciuri*, *S. haemolyticus*, *S. epidermidis*, *S. simulans* and *S. auricularis* were the most frequently recovered CoNS species in blood cultures. They were present at 39%, 11%, 9.8%, 9.8%, 9.8% and 6.1% and 5% in the blood cultures, respectively. This is in consistence with other previous studies as Shruthi et al. (2015) who reported that the commonest isolates of CoNS isolated from blood were *S. hominis* with percentage reaches (48.4 %).

In our study, the rate of Oxacillin resistance among all isolated Staphylococci strains was (100%) in both *S. aureus* and CoNS isolates. These findings are consistent with Perveen et al. (2013) who observed that all *S. aureus* isolates were resistant to Oxacillin (100%) and (95.65 %) CoNS isolates were resistant to Oxacillin.

Similar high rates of Oxacillin resistance among neonatal CoNS isolates have been previously reported as 87% of strains were MRCoNS (Hira et al., 2007). In addition, in a study conducted at the Gulhane Military Medical Academy Hospital, Turkey, the overall methicillin

resistance was identified as 83.3% in 313 CoNS isolates (Mert et al., 2011). In other studies in Turkey, these rates were found to vary from 67.5% to 85% (Celebi et al., 2007 and Koksai et al., 2009). Intermediate rates were reported in Brazil (Pereira & Cunha Mde, 2013), in which the rate of Oxacillin resistance among the 100 CoNS strains isolated from neonatal blood cultures was 69%. Another study reported that MRCoNS rates in clinical samples vary between 55% and 77% (Piette & Verschraegen, 2009). These rates have even been reported as high as 86% in intensive care units.

This study further found all isolates of CoNS resistant to multiple antibiotics tested. Isolates exhibited resistance towards various antibiotics such as Cephalosporins, Pencillins, and Gentamicin, which is almost similar to previous reports (Ang et al., 2004). Another study James & Reeves (1996) found that CoNS strains resistant to first, second, third and fourth generation Cephalosporins. Perveen et al. (2013) reported that 86.95% resistance of CoNS against first generation Cephalosporins which is similar to our findings.

Gentamicin is an aminoglycoside and is most often prescribed because of its low cost and synergistic activity with β -lactum antibiotics (Lewis & Salyers, 2008). In the present study 59.8% of MRCoNS showed resistance towards Gentamicin which is similar to Olowe et al. (2007) where he reported 68% of MRCoNS resistance to Gentamicin.

Rifampicin is a drug considered suitable for treatment of MRSA infection (Bayer & Lam, 1985; Chambers et al., 1997 and Zavasky & Sande, 1998). In this study MRSA resistance to Rifampicin is found 12% and that of MRCoNS is found 28%. Jeffrey et al. (2015) reported that MRSA resistance of 12% towards Rifampicin and that of MRCoNS is 6%. Other studies Mahmood et al. (2001) and Olowe et al. (2007) have reported that MRSA resistance of 14% towards Rifampicin.

In this study 83.3% of MRSA were resistant to Clindamycin, while 40.2% CoNS were resistant, which is comparable to previous reports Bahmani et al. (2013) where they detected that 30% of MRSA were resistant to Clindamycin and 25% CoNS were resistant to Clindamycin. In this study MRSA resistance to Imipenem is found 44.4% and that of MRCoNS is found 98.8%. Perveen et al. (2013) reported that MRSA resistance of 77% towards Imipenem and MRCoNS were 78%.

Among Fluoroquinolones, Ciprofloxacin and Ofloxacin were tested; the percentage resistance found MRCoNS was 67.1% for Ofloxacin and 57.3% for Ciprofloxacin, respectively. Previously reported resistance of Ciprofloxacin shows a similar type of pattern (Ang et al., 2004 and Perveen et al., 2013).

Moreover, because of widespread methicillin resistance among Staphylococci spp., the most frequent causative microorganism among neonates and empiric treatment of Staphylococcal infection with Vancomycin is advocated strongly in many neonatal wards (Kalantar et al., 2007 and Ghelbi, 2008). In our study, we observed that more than 12% resistance to Vancomycin in CoNS and in *S. aureus* 72%. The previous studies showed prevalence of *Staphylococcus* strain resistant to Vancomycin in Iran (Shahrbanoie, 2005). Also Ang et al. (2004) reported that the prevalence of Vancomycin resistance CoNS as all the isolates in that study showed resistance to Vancomycin. In Britain, France, United state reports of outbreak strains VRSA observed (Tenover & Lancaster 2001).

In our study, type I was the most common type being present in 33 of 50 methicillin resistant Staphylococci (MRS) species (66%) either alone or combined with other type, followed by type (II or III) (20/50) (40%). Difference in the methods used in SCCmec typing affects the interpretation of the data reported and renders comparison difficult among the different studies (Zhang et al., 2005). In other studies (Machado et al., 2007 and Pereira & Cunha Mde, 2013), SCCmec type (II or III) was the most prevalent followed by type I. However, their results cannot be compared with ours due to difference in the method and primers used.

Conclusion

The spectrum of children with sepsis as seen at Mansoura University Children Hospital (MUCH), confirmed the importance of pathogens such as *Staphylococcus* spp. Similar to other studies from the developing countries, CoNS species were the most common isolated pathogens in children with sepsis. Among CoNS isolates, *S. hominis* was the most predominant, followed by *S. sciuri*. All of Staphylococci septicemia was methicillin resistant Staphylococci so resistant strains were more common in Staphylococci isolates among our population. Prevalence of SCCmec types I and (II or III) which are typically associated with hospital acquired methicillin resistant Staphylococci (HA-MRS) isolates among children with sepsis.

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الفحص الظاهري و الوراثة لأنواع بكتيريا المكورات العنقودية المقاومة للميثيسيلين المعزولة من أطفال مصابه بتسمم الدم في مصر

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تعتبر بكتيريا المكورات العنقودية من المسببات الرئيسية في الإصابة بالتسمم الميكروبي في الدم . وهذه البكتيريا لها قدرة عالية على اكتساب المقاومة ضد المضادات الحيوية وهي بذلك تعتبر من أهم أنواع البكتيريا المسببة للإصابات داخل المستشفيات. لذلك تأتي هذه الدراسة كواحدة من المحاولات العلمية لدراسة هذه الظاهر هو معرفة أنواع سلالات بكتيريا المكورات العنقودية المقاومة للميثيسيلين المعزولة من الأطفال المصابين بالتسمم الميكروبي في الدم والكشف عن نسبه وجود هذه البكتيريا داخل مستشفى الأطفال الجامعي بمدينة المنصورة وكذلك معرفة مستوى مقاومة هذه البكتيريا لباقي المضادات الحيوية والتي تستخدم عادة لعلاج مثل هذا النوع من البكتيريا. كما تطرقت الدراسة أيضا لمعرفة المكون الوراثي لمقاومة بكتيريا المكورات العنقودية للميثيسيلين باستخدام تفاعل البلمرة المتسلسل (PCR).

و قد تم تعريف العزلات توزيعها إلى مجموعتين وهي بكتيريا المكورات العنقودية موجبه التخثر (S. aureus) وبكتيريا المكورات العنقودية سالبه التخثر حيث كانت نسبة كل منهما (18% و 82%) على الترتيب. و قد تبين أن بكتيريا *S. hominis* subsp. *hominis* كانت الأكثر شيوعا بين بكتيريا المكورات العنقودية سالبة التخثر بواقع 32 عزلة بنسبة 39% يليها بكتيريا *S. sciuri* بواقع 9 عزلات بنسبه 11%.

وقد أظهرت الدراسة أن جميع بكتيريا المكورات العنقودية (بكتيريا المكورات العنقودية موجبة وسالبة التخثر) كانت مقاومة للأوكساسيلين. وقد وجد من خلال هذه الدراسة أن العزلات التي احتوت على مكون جيني واحد كانت 27 عزله بنسبه (54%) سواء كان النوع الأول *SCCmec* او (النوع الثاني أو الثالث) *SCCmec* في حين أن 13 (26%) من العزلات المختارة احتوت على نوعين من المكون الجيني *SCCmec* كما أن 10 (2%) من العزلات المختارة لم يكن للمكون الجيني *SCCmec* موجود بها.

أن الكشف عن بكتيريا المكورات العنقودية المقاومة للميثيسيلين يلقي الضوء عن مدى انتشار هذه البكتيريا داخل مستشفى الأطفال الجامعي بمدينة المنصورة. لهذا توصي الدراسة بسرعة الكشف عن بكتيريا المكورات العنقودية المقاومة للميثيسيلين عند المرضى المقيمين بالمستشفى.