ORIGINAL ARTICLE

Resistance Pattern and Virulence Factors of CoNS Isolates from Sepsis Associated Acute Kidney Injury

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Key words:
Sepsis; AKI; CoNS; enterotoxin; slime

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ABSTRACT

Background*: Acute kidney injury (AKI) is among the most frequent complications of sepsis. Objectives: This study evaluated the antibiotic resistance pattern and virulence factors; slime production and staphylococcal enterotoxins genes of CoNS isolates recovered from blood of sepsis patients with AKI.* ***Methodology****: Blood samples were collected from patients diagnosed as sepsis and AKI from October 2018 to September 2020 in Mansoura University Main and New Generalized Hospitals. Blood cultures were done and the isolates were identified by MALDI-TOF Biotyper™. Antibiotic testing was done by the disc diffusion test for CoNS isolates. Slime production and staphylococcal enterotoxins, toxic shock syndrome toxin (tsst) and mec A genes were evaluated.* ***Results****: A total of 73 patients diagnosed as sepsis with AKI were enrolled in this research; 21 had positive blood cultures, with 66.7% Gram positive bacteria. All cases with Gram-negative bacterial blood culture were significantly complicated with chronic kidney disease compared to 42.9% of Gram-positive bacterial blood cultures (p = 0.01). Eleven out of 14 Gram-positive bacterial blood cultures were CoNS strains; seven S. haemolyticus, three S. epidermidis and one S. hominis strains. All CoNS isolates were sensitive to vancomycin and linezolid. Eight (72.7%) CoNS isolates had mecA gene. About 63.6% of CoNS strains were slime producers. Enterotoxin gene sec was the predominant among the isolates of CoNS (36.4%).* ***Conclusion:*** *S. haemolyticus was the most frequent isolated species from blood of patients with sepsis associated AKI. Majority of CoNS strains had mecA gene and were slime producers. Sec gene was the most often detected enterotoxin gene.*

INTRODUCTION

Sepsis results from the dysregulated host response to infection and is responsible for 45–70% of acute kidney injury (AKI) cases in patients with critical illness[1,2]. In sepsis-associated AKI, AKI manifests within 7 days after the onset of sepsis (as determined by the Kidney Disease Improving Global Outcome criteria and Sepsis 3 criteria, respectively)[3].

Sepsis-associated AKI is a life-threatening complication associated with high morbidity and mortality[4]. It results directly from the infection or the host reaction to infection or as an indirect side effect of sepsis or sepsis treatments[5].

Complications of sepsis-associated AKI include progression to acute kidney disease (AKD); persistently decreased kidney function and chronic kidney disease (CKD) that depend on the degree and length of AKI. The shorter the duration of AKI, the better the prognosis. Reversal of AKI is linked to improved patient survival[6].

Sepsis can result from several healthcare–associated or community–acquired microorganisms that are resistant to the commonly prescribed antimicrobial drugs[7]. Early, proper and monitored antibiotic therapy is a corner stone in the prevention of sepsis associated AKI and improvement of the prognosis of disease[4].

Data on the epidemiology of sepsis associated AKI in Egypt is limited. Sepsis associated AKI was common among ICU patients in Cairo[8] and Aswan[9], Egypt.

Coagulase-negative staphylococci (CoNS) is a member of the normal skin flora and mucosa that have caused many hospitals acquired infections. Among coagulase-negative staphylococci, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus* and *Staphylococcus hominis* are common cause of sepsis[10].

The virulence of CoNS depends on the biofilm formation that promotes the persistence of CoNS isolates to inanimate objects protecting them from

antibiotics and the immune response[11], besides the production of staphylococcal enterotoxins and toxic shock syndrome toxin 1 (*TSST-1*)[12,13]. Slime secretion helps the adherence of CoNS to the host cells, medical equipments and surfaces and protects CoNS from antibiotics, phagocytosis and chemotaxis[10].

Therapy for infections induced by CoNS is a challenge because of the rise in the antibiotic resistance especially to beta-lactam drugs and the multi-drug resistance of these isolates[10]. The *mecA* gene, carried on the mobile genetic island staphylococcal cassette chromosome *mec* (SCC*mec*), encodes the methicillin resistance and has been documented in about 80% of the CoNS strains causing sepsis[14].

The aim of this study was to evaluate the antibiotic resistance pattern and virulence factors; slime production and staphylococcal enterotoxins and toxic shock syndrome toxin *(tsst)* genes of CoNS strains retrieved from blood of patients with sepsis associated AKI.

METHODOLOGY

Study Population:

This is a cross sectional pilot study conducted on patients of Mansoura main and New Generalized Hospitals over a period of two years from October 2018 to September 2020.

The participants inclusion criteria were adult patients ≥18 years with sepsis identified according to the third international consensus definitions and evaluated by Acute Physiology and Chronic Health Evaluation II (APACHE II) score[15]. AKI was defined by the AKI study group; Kidney Disease Improving Global Outcomes (KDIGO)[16].

Exclusion criteria included a history of nephrectomy, documented end-stage renal disease and kidney transplantation, pregnant female, autoimmune diseases, pancreatitis, heat stroke, trauma, burns, surgery and malignancy.

The Institutional Research Board (IRB) of Mansoura Faculty of Medicine approved this study (R.23.08.2293) and each participant provided an informed consent before entering the study.

Sample Collection:

Blood samples were collected from sepsis patients with AKI under aseptic conditions[17]. Blood cultures were considered positive for CoNS isolates only when at least two blood cultures positive for CoNS were obtained within five days[18].

CoNS Isolates Identification:

The recovered isolates were phenotypically identified by the standard microbiological methods including the colony morphology, Gram staining and catalase and coagulase tests then species identification was further confirmed with the MALDI-TOF Biotyper™ (Bruker Daltonics)[19].

Antimicrobial Susceptibility Pattern:

Antibiotic sensitivity of the isolated CoNS was tested by the disc diffusion method on Mueller-Hinton agar (bioMérieux, Marcy l'Etoile, France) using the following antibiotics; ampicillin (10µg), amoxicillin-clavulanate (20/10µg), cefuroxime (30µg), vancomycin (30µg), linezolid (10µg), gentamicin (10µg), ciprofloxacin (5µg) and trimethoprim/sulfamethoxazole (1.25/23.7ug) (Oxoid Ltd, England)[20].

Slime Production Assay:

Qualitative detection of slime formation by CoNS isolates was examined by culturing the strains on sterile Congo red agar plates as previously described by Freeman *et al*[21].

Detection of *mecA*, Staphylococcal Enterotoxins and *tsst* Genes

DNA Isolation:

Genomic DNA was extracted from CoNS isolates using i-genomic BYF DNA Extraction Mini Kit (Interchim, France) as per the manufacturer's instructions.

Multiplex PCR:

Two sets of multiplex PCR was performed; one set for determination of staphylococcal enterotoxins genes (*sea*, *seb*, *sec*, *sed*) and the second set for *mec A* and toxic shock syndrome toxin (*tsst*-1) genes detection[22] using primers in table 1.

Table 1: Primers and product sizes of *mecA*, staphylococcal enterotoxins and *tsst* genes[22]

Gene	Primer Sequence	Product size
mecA	5′-ACTGCTATCCACCCTCAAAC-3′ 5′-CTGGTGAAGTTGTAATCTGG-3′	163
sea	5′-GGTTATCAATGTGCGGGTGG-3′ 5′-CGGCACTTTTTTCTCTTCGG-3′	102
seb	5′-GTATGGTGGTGTAACTGAGC-3′ 5′-CCAAATAGTGACGAGTTAGG-3′	164
sec	5′-AGATGAAGTAGTTGATGTGTATGG-3′ 5′-CACACTTTTAGAATCAACCG-3′	451
sed	5′-CCAATAATAGGAGAAAATAAAAG-3′ 5′-ATTGGTATTTTTTTTCGTTC-3′	278
tsst	5′-ACCCCTGTTCCCTTATCATC-3′ 5′-TTTTCAGTATTTGTAACGCC-3′	326

Statistical Analysis:

Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 16 (SPSS Inc, Chicago, IL, USA). Qualitative data were expressed as numbers and percentages. Quantitative data were presented as mean and standard deviation. The Chi-square or Fisher's exact test was employed to compare groups, as appropriate. Results with $p<0.05$ were considered statistically significant.

RESULTS

Demographic Characters of the Study Population

This research was carried out over two years and included 73 hospitalized patients clinically had sepsis associated AKI; females; 40 (54.8%) and males 33 (45.2%). Forty patients (54.8%) were from the rural areas. The median age was 60 years. The hospital stay ranged from 0-22 days.

Blood cultures were positive in only 21 out of 73 (28.8%) sepsis patients with AKI. Identifying positive blood cultures phenotypically revealed 14 (66.7%) Gram positive bacterial blood culture while Gram negative bacteria (*E. coli* and *Klebsiella species*) were isolated from 33.3% (No = 7) of positive cultures.

Positive bacterial blood cultures were commonly isolated from female patients (57.1%). While Gram positive bacteria were common among patients from urban areas (64.3%), Gram negative bacteria were common among patients from rural areas (57.1%), (table 2).

The most frequent comorbidity in Gram positive bacteremia was arterial hypertension (85.7%) and diabetes mellitus (78.6%), while arterial hypertension was a common risk factor (42.9%) in Gram negative bacteremia.

Most Gram-positive bacteremia cases (92.8%) were hospital acquired compared to 85.7% of Gram-negative bacteremia cases. All Gram-negative bacteremia cases were significantly complicated with chronic kidney disease (CKD) compared to 42.9% of Gram-positive bacteremia cases ($p = 0.01$). Mortality of septic AKI cases with Gram-negative bacteremia were high compared to cases with Gram-positive bacterial blood cultures (71.4% vs 28.6%), as shown in table 2.

Table 2: Demographic data, comorbidities and fate of patients with sepsis and AKI recovered Gram positive and Gram-negative bacteria from blood cultures.

	Gram Positive Bacterial Blood Culture No = 14 No (%)	Gram Negative Bacterial Blood Culture No = 7 No (%)	P value
Patients Demographics			
Age	53.8-69.6 (65)	48.2-70.6 (65)	0.5
Sex			
Male	6 (42.9%)	3 (42.9%)	1
Female	8 (57.1%)	4 (57.1%)	
Residence			
Urban	9 (64.3%)	3 (42.9%)	0.39
Rural	5 (35.7%)	4 (57.1%)	
Comorbidities			
Arterial hypertension	12 (85.7%)	3 (42.9%)	0.12
Diabetes mellitus	11 (78.6%)	2 (28.6%)	0.05
Ischemic heart diseases (IHD)	5 (35.7%)	2 (28.6%)	1
Chronic liver disease (CLD)	3 (21.4%)	2 (28.6%)	1
Hospital acquired (HA)	13 (92.8%)	6 (85.7%)	1
Fate			
chronic kidney disease (CKD)	6 (42.9%)	7 (100%)	0.01
Dialysis	5 (35.7%)	3 (42.9%)	1
Death	4 (28.6%)	5 (71.4%)	0.15

Species identification using the MALDI-TOF Biotyper™ revealed that eleven out of 14 Gram-positive bacterial blood cultures were CoNS strains; seven *S. haemolyticus*, three *S. epidermidis* and one *S. hominis* strains and the other three strains were enterococci.

Antibiotic susceptibility of the CoNS isolates was examined by the disc diffusion test and revealed that all strains were susceptible to vancomycin and linezolid. Most of CoNS isolates resisted trimethoprim/ sulfamethoxazole (91%). Resistance to ampicillin, amoxicillin-clavulanate, cefuroxime, ciprofloxacin and gentamicin were 72.7%, 72.7%, 72.7%, 54.5% and 45.5%, respectively. Resistance to β-lactam drugs was highest among *S. hominis* then *S. haemolyticus* followed by *S. epidermidis* isolates, (table 3).

Table 3: Antimicrobial resistance pattern of CoNS strains recovered from blood cultures of patients with sepsis and AKI

	S. haemolyticus **No = 7 No/%**	***S. epidermidis*** **No = 3 No/%**	**S. *hominis*** **No = 1 No/%**	**Total No = 11 No/%**
Vancomycin	0	0	0	0
Linezolid	0	0	0	0
Trimethoprim/sulfamethoxazole	6 (85.7%)	3 (100%)	1 (100%)	10 (91%)
Cefuroxime	5 (71.4%)	2 (66.7%)	1 (100%)	8 (72.7%)
Ampicillin	5 (71.4%)	2 (66.7%)	1 (100%)	8 (72.7%)
Amoxicillin-clavulanate	5 (71.4%)	2 (66.7%)	1 (100%)	8 (72.7%)
Ciprofloxacin	3 (42.9%)	2 (66.7%)	1 (100%)	6 (54.5%)
Gentamicin	3 (42.9%)	2 (66.7%)	0	5 (45.5%)

Eight (72.7%) CoNS isolates; 5 *S. haemolyticus* (71.4%), 2 *S. epidermidis* (66.7 %) and 1 *S. hominis* strains had *mecA* gene detected by multiplex PCR, (table 4).

On Congo red agar plates, seven (63.6%) out of eleven CoNS isolates; 6 S. *haemolyticus* (85.7%) and 1 *S. epidermidis* (33.3%) strains produced slime layer, (table 4).

Using multiplex PCR, only one (14.3%) *S. haemolyticus* strain had staphylococcal enterotoxin genes *sea*, *seb*, *sec* and *sed* and all three *S. epidermidis* isolates had *sec* enterotoxin gene. Only two *S. haemolyticus* strains (28.6%) had *tsst* gene, (table 4).

Table 4: Distribution of slime production and enterotoxins, *tsst* and *mecA* genes among CoNS isolates recovered from blood cultures of patients with sepsis and AKI.

	S. haemolyticus **No = 7 No/%**	***S. epidermidis*** **No = 3 No/%**	***S. hominis*** **No = 1 No/%**	**Total No = 11 No/%**
Slime production	6 (85.7%)	1 (33.3%)	0	7 (63.6%)
Enterotoxin genes				
Sea	1 (14.3%)	0	0	1 (9.1%)
Seb	1 (14.3%)	0	0	1 (9.1%)
Sec	1 (14.3%)	3 (100%)	0	4 (36.4%)
Sed	1 (14.3%)	0	0	1 (9.1%)
Tsst	2 (28.6%)	0	0	2 (18.2%)
mecA	5 (71.4%)	2 (66.7%)	1 (100%)	8 (72.7%)

DISCUSSION

Sepsis is considered a heterogenous syndrome caused by several microorganisms affecting multiple vital organs. It is linked to high morbidity and death worldwide and the rapid diagnosis and early antibiotic therapy and resuscitation are mandatory to reduce the associated organ damage and improve the outcome[23, 24]. Sepsis is a leading cause of AKI in patients with critical illnesses and its rate increases worldwide[25].

This study involved 73 patients with AKI and sepsis where patients' blood cultures were positive in 28.8% of cases; 66.7% of cases recovered Gram positive bacteria mainly CoNS isolates (78.6%) and 33.3% of cases

yielded Gram negative bacteria. Gram positive bacteremia was common in females from urban areas.

Our data are in accordance to another study where the rate of positive blood culture from sepsis patients was 35% and Gram-positive bacteria were the predominant causative microorganism of sepsis mainly CoNS[26]. However, our data are higher than another study from Korea where positive blood cultures was 47% in patients with sepsis mainly due to *E. coli* and *Klebsiella species* rather than *Staphylococcus* and *Streptococcus species*[24].

Blood culture is mandatory for diagnosis and treatment of sepsis, yet it is often negative in cases with suspected sepsis. It has been documented that about 50% of patients with serious sepsis are culture-negative[27].

Reasons for the blood culture negativity might be due to empirical antibiotic treatment within previous 48 hours and the sepsis might be due to non-culturable pathogens; viruses or fungi or noninfectious causes; metabolic disorders, inflammatory diseases, side effects of drugs or malignancies[24].

Conventional microbiological techniques might not identify some microorganisms due to technical issues or intrinsic causes linked to the microorganisms, while molecular techniques could rapidly identify microorganisms in culture negative blood cultures[28].

The most prevalent comorbidity in patients with bacteremia was arterial hypertension in this study consistent with previous studies[16,25,29].

All Gram-negative bacterial blood culture cases were significantly complicated with CKD compared to Gram-positive bacterial blood culture cases. The mortality of Gram-negative bacteria septic AKI cases was higher than Gram-positive bacteria sepsis associated AKI cases (71.4% vs 28.6%). It's been documented that septic AKI patients with Gram negative bacterial blood cultures were more serious, had a higher disease severity score and had shorter time to develop into AKI[30]. Additionally, in patients with bacteremia, the mortality rate was more in patients with Gram-negative sepsis compared to those with Gram-positive sepsis as the later patients might receive effective empirical antibiotics treatment compared to patients with Gram-negative pathogens[31].

Staphylococcus haemolyticus followed by *S. epidermidis* and *S. hominis* were the most common species isolated from blood culture of sepsis patients with AKI in this study as identified by the MALDI-TOF Biotyper™. The same was reported by an African study where *S. haemolyticus* was the leading cause of bloodstream infection then *S. epidermidis* and *S. hominis*[7]. Additionally, a Nigerian study investigated the species distribution from 105 CoNS isolates documented the high incidences of *S. haemolyticus* followed by *S. epidermidis*[32]. However, *S. epidermidis* was the most frequent CoNS isolates from bacteremia in other studies then *S. haemolyticus* and *S. hominis*[10,33,34,35].

In this research, all CoNS isolates were sensitive to vancomycin and linezolid. Resistance to β-lactam drugs was high (72.7%) especially among *S. hominis* then *S. haemolyticus* and *S. epidermidis* isolates. The *mecA* gene was recovered in eight (72.7%) CoNS isolates. This high level of resistance to β-lactams was recorded in an African study from Zimbabwe (75%)[33], which also stated a similar level of resistance to ciprofloxacin (54.5%) and gentamicin (45.5%) and complete sensitivity to vancomycin and linezolid.

Similar to our study, the resistance was highest among S. *hominis, S. haemolyticus* then *S. epidermidis* in Benin, Africa[7]. Similar data was recovered from Malaysia[35] and Nepal where *mecA* gene encoding for penicillin-binding protein (PBP 2a) was recovered in 70.7% of the strains[36].

A reduced resistance to methicillin was recorded in Turkey; 67.5%[10] and 62%[37], while a greater degree of resistance was found in Saudi Arabia (90.7%)[38], Kuwait (92.4%)[12] and Jordan (93.6%)[33]. This difference in the incidence of antibiotic resistance might be due to different geographic areas and studied groups.

Slime production is associated with the pathogenicity as it helps the adherence and colonization of CoNS strains and acts as a reservoir of antibiotic resistance in hospital[10]. About 64% of CoNS isolates produced slime layer in our study mainly *S. haemolyticus.* On contrary, other studies[10,39]. documented a low incidence of slime production among CoNS isolates being predominant among *S. epidermidis* strains which might be due to different species distribution in our study.

In our research, *sec* enterotoxin gene was the predominant detected gene (36.4%) followed by *tsst* gene (18.2%). This is in agreement with many studies where *sec* gene was the most frequent detected enterotoxin gene between CoNS isolates and *tsst* gene was recovered in about 25% of the isolates[7,13,39]. Nevertheless, it has been stated that the dominant CoNS enterotoxin gene differs with the geographic locality[12].

This study had some limitations; blood cultures were tested only for bacterial growth not for viral or fungal growth, besides being a single-center study must be replicated with a large population within many centers before its generalization.

CONCLUSION

Staphylococcus haemolyticus was the most frequent isolated species from bacteremia of sepsis patients with AKI. Majority of CoNS isolates had *mecA* gene and were slime producers. The *sec* gene was the frequent detected enterotoxin gene. Adjustment of blood culture as a rapid diagnostic tool for sepsis is mandatory to optimize monitored early antibiotic treatment, decrease

the antibiotic resistance, decrease the rate of sepsis associated AKI and improve the outcome.

Declarations:
Consent for publication: Not applicable
Availability of data and material: Data are available upon request.
Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.
This manuscript has not been previously published and is not under consideration in another journal.
Funding: Authors did not receive any grants from funding agencies.

Authors' contributions:
Raghdaa Shrief and Rasha El-Mahdy contributed equally to this work. Rasha El-Mahdy and Raghdaa Shrief did the experiments and wrote the manuscript. Mohamed Sobh, Mohammed Kamal and Yara Moheb collected, assembled and analyzed clinical data. All authors reviewed the manuscript.

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