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Original article

Linezolid resistance in coagulase negative *Staphylococci* isolates and the related genes in intensive care unit patients in a University Hospital in Egypt

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ABSTRACT

Background: Coagulase-negative *Staphylococci* (CoNS) are opportunistic pathogens causing severe hospital-acquired infections. This study aimed to determine the frequency of linezolid-resistant CoNS (LRCoNS) in intensive care unit (ICU) infected patients and the related resistance genes. **Methods:** Seventy CoNS were isolated from 254 different clinical samples from ICU patients. They were identified by conventional methods; species were identified by API. Antimicrobial susceptibility test (AST) by disc diffusion method was performed for CoNS isolates. Methicillin resistance was identified by resistance to cefoxitin (30 µg) disc. Linezolid resistance was confirmed by measuring the minimal inhibitory concentration (MIC) using E-test strips (0.016-256 µg/ml). Three resistance genes (cfr, optrA and poxtA) were tested for the LRCoNS by PCR. **Results:** Among the 70 CoNS isolates, three LRCoNS were detected by disc diffusion method and confirmed by MIC (>256 µg/ml). Approximately 71.4% of the isolated CoNS were multi-drug resistant (MDR) and 68.6% were methicillin-resistant (MR). The three LRCoNS isolates (2 *Staphylococcus (S.) epidermidis* and one *S. haemolyticus*) were positive for cfr gene and negative for optrA and poxtA genes. **Conclusion:** The presence of cfr gene in the three LRCoNS isolates could explain the MDR of the three strains. This is considered an alarm for the antimicrobial resistance to the last resort antibiotics in hospital settings. So far, the situation is not threatening. Yet, continuous monitoring is essentially required.

Introduction

Coagulase-negative *Staphylococci* (CoNS) were regarded to be innocuous commensals due to their widespread colonization of human skin and mucosal membranes [1]. Currently, CoNS have become a classic opportunistic pathogen [2] with a significantly growing effect on human health and life as they are considered an important cause of nosocomial infections. *S. epidermidis*, *S. haemolyticus*, and *S. hominis* are the most prevalent bacteria that colonize human skin [3, 4]. They are recognized as major contributors to surgical-site infections (SSI), central line-associated bloodstream

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infections (BSI) [5, 6] and neonatal infections, including bacteremia [7].

CoNS are known for their capacity to produce antibiotic resistance to classes of routinely used antibiotics like β-lactams, macrolides, and aminoglycosides with remarkably high reported methicillin resistance rates [8] in addition to resistance to antibiotics of last chance such as the glycopeptides [9]. Consequently, the treatment of CoNS infections is difficult due to limited therapeutic options as a result of accompanying risk factors and multi-drug resistance (MDR) [10]. Linezolid, an oxazolidinone antibiotic, binds to the 50S ribosomal subunit and prevents the 70S ribosome formation which results in the suppression of the protein synthesis initiation. Linezolid binds a deep cleft of the 50S ribosomal subunit surrounded by 23S rRNA nucleotides [11].

In clinical settings, it is used to treat severe infections caused by Gram-positive bacteria that are resistant to antibiotics, including methicillin-resistant Staph. aureus (MRSA), methicillin-resistant CoNS, penicillin-resistant *Streptococcus pneumoniae*, and vancomycin-resistant *Enterococci* (VRE) [12]. Linezolid was used off-label to treat CoNS-caused meningitis [13], ventriculitis [14], osteomyelitis [15], and prosthetic-joint infections [16], even though it is not approved for the treatment of CoNS infections. However, oxazolidinone resistance develops when consumed in high doses or used for prolonged therapeutic courses, particularly in deeply seated infections [17].

In 2001, the first case of linezolid resistance was reported in staphylococci clinical isolates [18]. Thereafter, MRSA strains that are resistant to linezolid [19-21] as well as CoNS strains that are resistant to linezolid, have been identified in healthcare settings more often [22, 23]. The main mechanisms of oxazolidinone resistance in CoNS are (A) 23S rRNA methylation [plasmid-borne chloramphenicol– florfenicol resistance (cfr) gene], (B) mutations in 23S rRNA and ribosomal proteins (rpl genes), and (C) efflux (plasmid-borne oxazolidinone phenicol transferable resistance (optrA) gene) [24, 25].

The chloramphenicol-florfenicol resistance (cfr) gene causes non-mutational oxazolidinone resistance. The cfr gene encodes a ribosomal methyltransferase thereby conferring cross-resistance to oxazolidinones, phenicols, pleuromutilins, lincosamides, and streptogramin A. (PhLOPSA phenotype) [26]. The cfr gene is carried by plasmids with a mobile function, resulting in horizontal spread within the genus of Staphylococcus, causing outbreaks of infection with resistant bacteria [19]. The peculiar transferable oxazolidinone resistance gene, optrA, unlike the cfr gene, exclusively provides cross-resistance to oxazolidinones such as tedizolid and phenicols. Moreover, a multi-resistance plasmid containing both cfr and optrA was found, subsequently reducing the effectiveness of oxazolidinone antibiotics [27]. The new gene phenicols oxazolidinones tetracycline (poxtA), which was revealed by Antonelli and his colleagues in 2018, confers transferable resistance to linezolid in MRSA strains. The poxtA gene encodes one of the ribosomal protection proteins, an ABC transporter protein, that is involved in antimicrobial resistance. The poxtA gene is closely linked to optrA and can influence sensitivity to phenicols, oxazolidinones, and tetracyclines. Furthermore, it was also discovered that poxtA gene could raise the level of resistance to oxazolidinone by acting in a synergistic way with other mechanisms of resistance [28].

To our knowledge, there have been reports of linezolid resistance among CoNS (LRCoNS) from several nations, including Northern American countries (USA, Mexico), Southern American countries (Brazil), European countries (Greece, Spain, Italy, France, and Ireland), and Asian countries [29]. However, scarce data exists in Zagazig University Hospitals in Egypt. Therefore, in the current study, we aimed to investigate the prevalence, identify the species, determine the antimicrobial susceptibility profile and detect the linezolid resistance genes among LRCoNS isolates obtained from ICU patients in Zagazig University Hospitals, Egypt.

Methods

This cross-sectional study was operated in the Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University from July 2022 to Janurary 2023. It included 254 samples that were aseptically collected at random from ICU patients at Zagazig University Hospitals. Sample size was calculated using Epi software version 6 at confidence interval 95%. Clinical samples were taken from all body sites where there was a possibility of CoNS infections. Patients were deemed eligible after fulfilling the subsequent inclusion criteria: Nosocomial

infections, immuno-compromised patients, and previous laboratory data reporting CoNS. Meanwhile, the exclusion criteria were: Patient unwillingness, previous laboratory examination determining bacteria other than CoNS, and good response to antibiotic therapy. A thorough clinical history was obtained. Patients' reports, which involved the diagnosis, any prior antibiotic therapy, and findings of past laboratory tests, were taken into consideration.

Ethical consideration

The study procedures were carried out in accordance with the Declaration of Helsinki. The Institutional Review Board of the [Faculty of Medicine, Zagazig University] approved the study (No: 9642-19-7-2022).

Patients' relatives were informed of the study's nature and goals before providing their written consent. Study participants were not subjected to any risk or injury. Patients' information was handled discreetly.

Bacterial isolation and identification

Seventy CoNS isolates were identified by conventional methods which include colony morphology on nutrient agar and blood agar, Gram stain, catalase test, and tube coagulase test [30]. Species identification was performed using API® Staph (bioMérieux Industry, USA) based on the manufacturer's instructions.

Antimicrobial susceptibility testing (AST)

CoNS isolates was tested for penicillin (10 µg), cefoxitin (30 µg), ciprofloxacin (5 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), clindamycin (2 µg), erythromycin (15 µg), doxycycline (30 µg), gentamicin (10 µg) and linezolid (30 µg/ml) (Oxoid Ltd., UK) by modified Kirby-Bauer disc diffusion method. Linezolid resistance was confirmed by measuring the minimum inhibitory concentrations (MICs) using the E-test strips (0.016-256 µg/ml) (Lioflichem s.r.I., Italy). Methicillin-resistant CoNS (MR-CoNS) was identified by resistance to cefoxitin (30 µg) disc. The results of the AST were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines, 2022 [31].

Detection of linezolid resistance genes

DNA extraction

All bacterial DNA was extracted using QIAamp DNA Mini kit (Qiagen, N.V.) from 2 ml of overnight grown bacterial cultures in nutrient broth then the protocol for isolation of Gram-positive bacterial DNA was followed as per the manufacturer's instructions. DNA concentration was determined by UV spectrophotometry at 260 nm, readings between 0.1 and 1.0 were considered acceptable. The DNA purity was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. A260/A280 ratio for pure DNA ranges from 1.7 to 1.9.

Polymerase chain reaction

Standard PCR was performed using PCR Master Mix (2X) (Thermo Scientific, Ca, USA) according to the manufacturer's instructions to detect the plasmid-transmitted genes (cfr, optrA, and poxtA) in linezolid-resistant CoNS isolates. The primers used for the amplification of these genes are listed in **table (1).** The thermal profile for amplification of cfr and poxtA genes was set as follows; initial denaturation at 95°C for 1 min, followed by 40 cycles of denaturation at 95° C for 30 sec, annealing at 68° C for 30 sec, extension at 72° C for 1 min and final extension at 72° C for 10 min. Meanwhile, the annealing temperature used with the optrA gene was set at 57°C, otherwise, the PCR conditions were the same as previously mentioned.

Statistical analysis

The collected data were coded, entered, presented, and analyzed by computer using a database software program, Statistical Package for Social Science (SPSS) version 26. Qualitative data were represented as frequencies and percentages and the Chi-square test was used for the analysis. For quantitative variables, mean, standard deviation (SD), median, and (minimum-maximum) were computed.

Table 1. Primers used in PCR reaction.

Gene	Primer sequence	Reference
cfr	**Forward**: 5'TGA AGT ATA AAG CAG GTT GGG AGT CA3'	(29)
	Reverse: 5'ACC ATA TAA TTG ACC ACA AGC AGC3'	
poxtA	**Forward:** 5'TCA GAG CCG TAC TGA GCA AC3'	(35)
	Reverse: 5'CGT TTC TGG GTC AAG GTG GT3'	
optrA	**Forward**: 5'AGG TGG TCA GCG AAC TAA3'	(25)
	Reverse: 5'ATC AAC TGT TCC CAT TCA3'	

Results

Demographic characteristics of the studied patients and the distribution of isolated CoNS in the clinical specimens

The patients' characteristics are shown in **table (2)** as the mean age was 38.09±13.83 years with a median is 31 (27-52) years. More than half of the studied patients were males (58.6%). Among the 254 clinical isolates, 70 (27.6%) were identified as CoNS by positive Gram stain, positive catalase test, and negative coagulase test [35]. As demonstrated in **figure (1),** most of the CoNS isolates, 27 (38.6%), were isolated from SSI specimens. The most frequently isolated CoNS species were *S. epidermidis* (32/70, 45.7%), *S. haemolyticus* (26/70, 37.1%) and *S. hominis* (9/70, 12.9%). *S. saprophyticus* (2/70, 2.9%), *S. capitis* (1/70, 1.4%) were less frequently isolated.

Antimicrobial susceptibility profile of CoNS isolates

The antibiotic susceptibility profile of CoNS isolated strains was analyzed and presented in **(Figure 2).** All the studied CoNS isolates were resistant to penicillin (100%). A high frequency of resistance (68.6%) was detected with cefoxitin representing the MR-CoNS isolates **(Table 3),** followed by trimethoprim-sulfamethoxazole (44.3%), gentamycin (42.9%), erythromycin (38.6%), clindamycin (30%) then ciprofloxacin (25.7%). Furthermore, the lowest percentage of CoNS isolates (4.3%) were resistant to linezolid and doxycycline. However, linezolid was the most effective antimicrobial for most isolates with 95.7% sensitivity. Linezolid resistance was confirmed by measuring MIC values for all the three LRCoNS isolates which were more than 256 µg/ml as shown in **figure (3).** Among the three LRCoNS isolates, two strains were identified as *S. epidermidis* strains and one was identified as *S. haemolyticus*.

Multi-drug resistant CoNS and methicillin resistant-CoNS

More than half of the isolated CoNS (71.4 %) were MDR (defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories) [32] as displayed in **table (3).** MDR is a known hallmark and major issue, particularly in nosocomial and infection-associated CoNS [3]. There was a statistically highly significant association between the percentage of MDR-CoNS and the clinical specimens, $p<0.05$ as presented in **table (4).** The highest percentage of the MDR-CoNS were isolated from SSI (50 %), followed by ETA specimens (32 %). Meanwhile, the lowest percentages of the MDR-CoNS were isolated from blood and urine samples (10% and 8%, respectively). However, there was no statistically significant association between the MR-CoNS isolates and the clinical specimens, $p>0.05$.

Although all the three LRCoNS isolates were MDR, there was no statistically significant association between LRCoNS and MDR-CoNS, $p>0.05$. Additionally, only two LRCoNS isolates were MR-CoNS with no statistically significant relationship between both groups of isolates, $p>0.05$ **(Table 5).** The clinical data of the three patients from whom the three LRCoNS were isolated was described in **table (6).**

Determination of the resistance genes (cfr, poxtA, and optrA)

PCR analysis of the three LRCoNS isolates (2 S. epidermidis and one *S. haemolyticus*) for detection of the plasmid transmitted resistance genes, revealed that all of them were positive for cfr gene as shown in **figure (4).** On the other hand, all the three

LRCoNS isolates were negative for poxtA and optrA genes.

Table 2. Demographic data of the CoNS-infected patients (n=70).

Characteristic	Category	Study group (n=70)	
		No.	%
Gender:	**Female**	29	41.4
	Male	41	58.6

SD: Standard Deviation, IQR: Interquartile Range

Table 3. Frequency distribution of the MR and MDR among CoNS isolates (n=70).

Bacteria			
		Negative	Positive
MR-CoNS	N	22	48
	%	31.4	68.6
MDR-CoNS	N	20	50
	%	28.6	71.4

MR-CoNS: Methicillin-resistant coagulase negative *Staphylococci*, MDR-CoNS: Multidrug resistance bacteria coagulase negative *Staphylococci*.

Table 4. Distribution of the isolated MR-CoNS and MDR-CoNS from different clinical specimens (n=70).

Variables			MR-CoNS		X^2	*P* value
			Negative	Positive		
Specimen	**Blood**	N	6	8	7.1	0.067
		%	27.3%	16.7%		
	ETA	N	3	16		
		%	13.6%	33.3%		
	SSI	N	7	20		
		%	31.8%	41.7%		
	Urine	N	6	4		
		%	27.3%	8.3%		
	Total		22	48		
			MDR-CoNS		X^2	*P* value
			Negative	**Positive**		
Specimen	**Blood**	N	9	5	20.03	<0.001*
		%	45.0%	10.0%		
	ETA	N	3	16		
		%	15.0%	32.0%		
	SSI	N	2	25		
		%	10.0%	50.0%		
	Urine	N	6	4		
		%	30.0%	8.0%		
	Total		20	50		

X^2 = Chi square test, *p<0.05 is statistically significant, Methicillin-resistant coagulase negative staphylococci (MR-CoNS), Multidrug resistance bacteria coagulase negative staphylococci (MDR-CoNS), ETA : Endotracheal aspirate, SSI: Surgical site Infection

Table 5. Relation between linezolid sensitivity and both MR-CoNS and MDR-CoNS.

			Linezolid Sensitivity		X^2	*P* value
			Resistant (n=3)	Sensitive (n=67)		
MR-CONS	**Negative**	N	1	21	0.005	0.942
		%	33.3%	31.3%		
	Positive	N	2	46		
		%	66.7%	68.7%		
MDR-CoNS	**Negative**	N	0	20	1.43	0.231
		%	0.0%	29.9%		
	Positive	N	3	47		
		%	100.0%	70.1%		

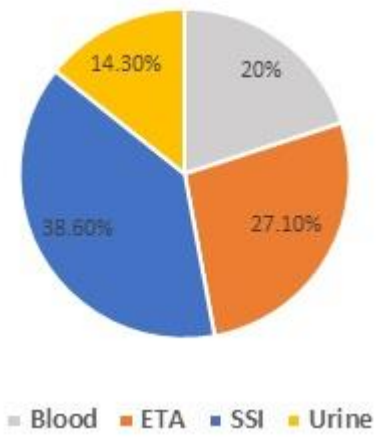
X^2 = Chi square test, Methicillin-resistant coagulase negative *Staphylococci* (MR-CoNS), Multidrug resistance bacteria coagulase negative *Staphylococci* (MDR-CoNS)

Table 6. Clinical data of the three LRCoNS infected patients.

	Patient No.1	**Patient No. 2**	**Patient No.3**
Age (years)	36	47	52
Gender	Male	Male	Female
Type of infection	Cancer colon with catheter associated urinary tract infection	Surgical site infection after diabetic foot amputation	Stroke with septicemia
Sample	Urine	Pus	Blood
Previous intake of linezolid	No	No	Yes
Previous hospitalization	Yes	Yes	Yes
Species of isolated CoNS	*S. epidermidis*	*S. epidermidis*	*S. haemolyticus*
MR\MDR- CoNS	MDR- CoNS	MR-CoNS and MDR-CoNS	MR-CoNS and MDR-CoNS

MR-CoNS: Methicillin-resistant coagulase negative *Staphylococci*, MDR-CoNS: Multidrug resistance bacteria coagulase negative *Staphylococci*.

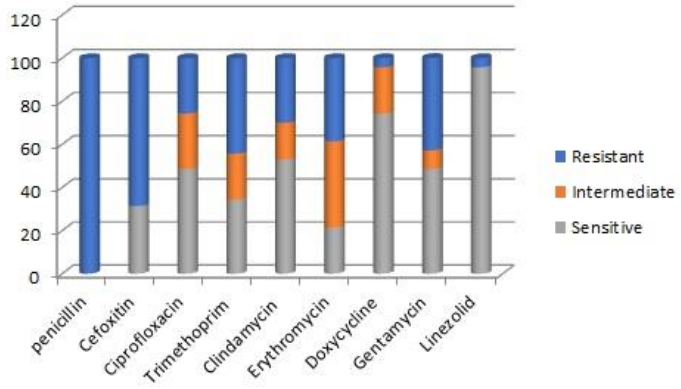
Figure 1. Distribution of CoNS isolates among different clinical samples.



SSI: blue color, ETA: orange color, blood: grey color, urine: yellow color.

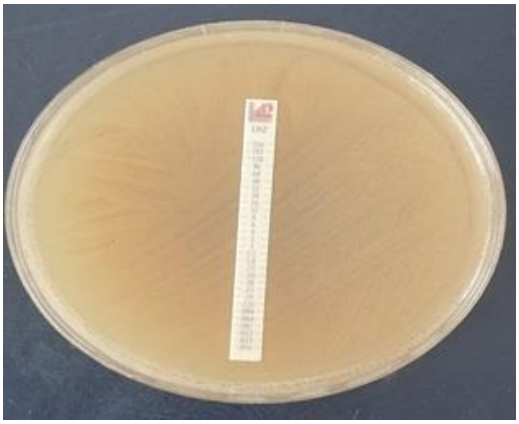
Seventy clinical CoNS isolates were detected in ICU patients according to the type of their infection. Surgical site infection (SSI) represented (38.6%) followed by endotracheal aspirates (ETA) specimens (27.1%), blood (20%), then urine representing (14.3%).

Figure 2. Antibiotic susceptibility profile of CoNS isolates.



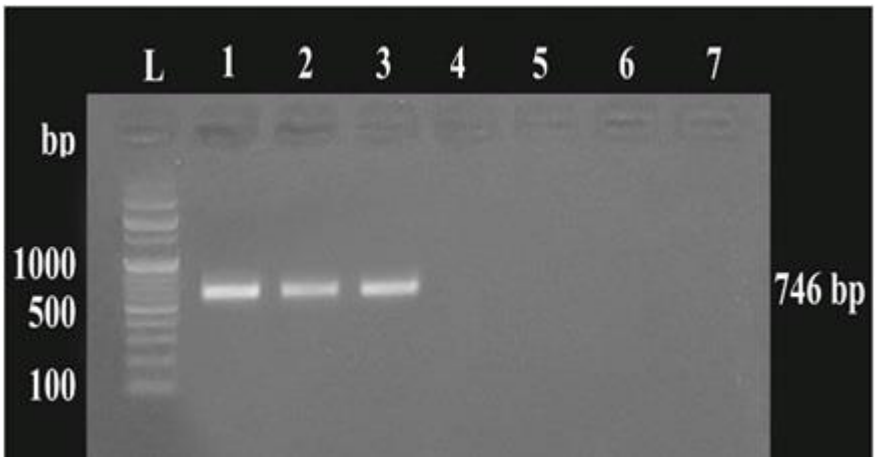
Blue color indicates resistant strains, orange color indicates intermediate sensitivity, and grey color indicates sensitive strains. Antibiotic susceptibility of the 70 CoNS isolates was determined by disc diffusion method. All the examined isolates were resistant to penicillin (100%). A high frequency of resistance (68.6%) was detected with cefoxitin followed by trimethoprim-sulfamethoxazole (44.3%), gentamycin (42.9%), erythromycin (38.6%), clindamycin (30%), ciprofloxacin (25.7%). Linezolid and doxycycline showed the least resistance percentage (4.3%). Linezolid was the most effective antimicrobial agent on majority of the isolates (91.4% sensitivity).

Figure 3. Minimum inhibitory concentrations (MICs) of LR-CoNS isolates.



Linezolid resistance was confirmed by measuring MIC of LR-CoNS using the E-test strips (0.016-256 µg/ml). This representative figure shows that the MIC of one isolated LR-CoNS was more than 256 µg/ml.

Figure 4. PCR products for LRCoNS with *cfr* gene-based primers.



This representative agarose gel electrophoresis figure shows that cfr gene amplified from the three LRCoNS isolates in lanes (1,2,3), the product size is 746 bp. L is marked for DNA ladder, 100 bp.

Discussion

CoNS are classic commensals of the skin and mucus membrane [3]. They act as opportunistic pathogens that cause serious healthcare-associated infections. They can cause human colonization and infection using variable methods, including adhesion, encroachment, endurance, and escape of the immune system [33]. It has been reported that immune-compromised patients, premature neonates, elderly patients, patients with multiple illnesses or chronic diseases, and patients who use invasive devices are more frequently to be prone to CoNS infections [3, 29].

In the present study, we have identified 70 (27.6%) CoNS isolates from 254 clinical specimens obtained from ICU patients in a university hospital in Egypt. A lower prevalence of CoNS isolated from healthcare associated infections (HAIs) (12.6% and 20.55%, respectively) was declared by previous studies in Egypt [34, 35]. We found that the high isolation rate of CoNS was from SSI specimens followed by ETA, blood then urine specimens. In accord with this finding, **Deyno et al.** reported that high CoNS isolation was from SSI specimens followed by urine specimens [36]. However, another 2 studies isolated CoNS more frequently from blood samples rather than other clinical samples [34, 37]. This distinction between our study and others could be explained by the variation in sample sizes, patient characteristics, and the isolated species. The most frequently isolated CoNS species in this study were *S. epidermidis, S. haemolyticus* and *S. hominis* (45.7%, 37.1% and 12.9%) respectively. This finding is in agreement with **Chaturvedi1 et al.** as they detected that most frequently isolated CoNS species was S.epidermidis (44.33 %), followed by S. haemolyticus (39.37 %) [37] and Nicolosi et al that reported that the most frequently detected CoNS were *S. haemolyticus , S. epidermidis* and *S. hominis* (47.1, 29.8, 7.8%) respectively [38].

CoNS infections can be especially challenging to treat since nosocomial CoNS are well known for quickly developing several resistance characteristics leading to MDR toward many routinely used antibiotics [38, 39]. Therefore, in this study, the antibiotic-resistance profiles of isolated CoNS were evaluated. All the isolated strains were resistant to penicillin (100%) which is similar to previous reports [8,40,41]. A high frequency of CoNS isolates (68.6%) was MR, mostly isolated from SSI and ETA specimens. This finding is in agreement with the other studies [38], [40], whereas other researchers showed a higher prevalence of MR-CoNS (79.8%, 77.63%, and 76.4%, respectively) [34], 41, 42]. It has been documented from many regions of the world that MR-CoNS are prevalent in hospitals [3,43]. However, the rise in antibiotic resistance in MR-CoNS isolated from hospitals makes this issue worse and presents a significant challenge for the management of HAIs [44].

Moreover, the resistance rate of other antimicrobials was observed in the isolated CoNS as follows; trimethoprim-sulfamethoxazole (44.3%), gentamycin (42.9%), erythromycin (38.6%), clindamycin (30%), ciprofloxacin (25.7%) and doxycycline (4.3%). Therefore, more than half (71.4%) of the isolated CoNS in our study were MDR. Due to the restricted access to newer antibiotics and the high expense of alternate therapeutics, MDR in CoNS is an issue in low- and middle-income countries [8]. A previous study in Egypt illustrated MDR-CoNS in ICU patients with high resistance to erythromycin (80%) followed by ciprofloxacin (66.45%), clindamycin (60.2%), gentamycin (51.3%) then doxycycline (43.47%) [34]. Furthermore, several studies confirmed the MDR of CoNS isolates with different patterns of antimicrobial resistance [38, 41]. A study described the incidence of MDR CoNS from different wards, including ICU, in three hospitals in South Africa. They reported high levels of antibiotic resistance rates for erythromycin (74.2%) and trimethoprim/sulfamethoxazole (68.5%) and high susceptibility to gentamicin (95.5%) with MDR phenotype (76.4%) [41]. Another one evaluated the antibiotic-resistance profiles of CoNS isolated from a hospital environment in South Italy as they demonstrated higher resistance to erythromycin followed by oxacillin, gentamycin, ciprofloxacin, then clindamycin [38]. The difference in the susceptibility profile between our study and others could be attributed to different strategies for antibiotic use in hospital settings. Furthermore, the limited use of certain antibiotics primarily for resistant staphylococcal infections may be the cause of the high sensitivity of these antibiotics [8].

Linezolid, an oxazolidinone antibacterial drug that inhibits protein synthesis [45], is a persuasive therapy for MDR Gram-positive bacteria and although it has been widely used for nearly 20 years, it still demonstrates outstanding action against Staphylococci [6]. However, there is an

alarming increment in linezolid-resistant CoNS [46]. In the current study, we detected three CoNS isolates exhibiting resistance to linezolid (4.3%). Nonetheless, linezolid sensitivity was the highest among all antibiotics tested (95.7%). Linezolid resistance in ICU patients may have developed as a result of the acquisition of strains carrying the linezolid resistance genes from their surroundings, a previous hospital stay, or linezolid therapy for infections [46]. Consistent with our study, previous studies reported the presence of linezolid resistance among CoNS isolates [29, 37, 41, 47-50]. Moreover, Maarouf et al reported that the prevalence of linezolid resistance among a collection of 232 clinical staphylococcal isolates obtained in 2011–2012 and 2015–2016 was established at 1.3% [51]. All three resistant isolates were identified as *S. haemolyticus*, which indicates a higher prevalence of linezolid resistance among CoNS than S. aureus [51]. In contrast to our finding, Nicolosi et al and Fahim et al did not record any LR-CoNS isolates in their study [34,38]. They explained this high sensitivity by the favored use of linezolid by hospital clinicians [38].

The development of linezolid resistance may be connected to the CoNS genome's remarkable plasticity, which is predominantly spurred by insertion sequences and other mobile genetic components (**Schoenfelder et al.** 2010). The cfr gene is typically found in a genetically unstable environment, either in the chromosome or on MDR plasmids [52]. Furthermore, cfr is plasmid-borne and frequently linked to transposons, which can lead to an adjusted interchange across Gram-positive bacteria and accessible transmission of cfr into vulnerable populations and other harmful bacteria [53]. Plasmid curing and the subsequent dramatic decrease of chloramphenicol and linezolid MICs by 16-and 64-folds, respectively, confirmed the role of cfr in linezolid resistance [51]. These findings agree with previously published studies that highlighted the role of cfr present on mobile element in linezolid resistance and the potential for its transfer from one isolate to another, increasing the prevalence of resistance [54]. Additionally, cfr was demonstrated in a 13 kilo base (kb) circular form, showing that the activity of insertion sequence (IS)1216 copies and flanking the region may facilitate recombination with other plasmids and increase cfr mobility [55]. Nevertheless, horizontal transmission of resistance is a serious threat, because the cfr gene can also be transmitted between species, such as from *S. epidermidis*, which although not pathogenic, could become a reservoir for resistance genes. **Morales et al.** showed that cfr-mediated resistance to linezolid was responsible for the first clinical outbreak of linezolid-resistant MRSA. The hospital isolated CoNS, which may be a reservoir of cfr-mediated resistance, could explain outbreaks [19].

In the present study, the three isolated LRCoNS (2 S. epidermidis and one *S. haemolyticus)* were positive for cfr gene. In line with our finding, several studies recorded the presence of cfr gene in isolated CoNS strains [29, 35, 46-48, 50, 56]. However, Maarouf et al reported that only one isolate among the three isolated LRCoNS (*S. haemolyticus*) carried the cfr gene [51]. It has been documented that cfr-mediated resistance restricts the range of the available antibiotics as it encodes resistance to a variety of them [47]. Hence, Staphylococci carrying cfr gene display an MDR phenotype, which agrees with the resistance profiles of our isolates [47]. We found that the three LRCoNS were MDR and 2 were MR with no statistically significant association between these groups, $p>0.05$. Meanwhile, the 3 isolated LRCoNS were negative for optrA and poxtA genes. In agreement with this record, Abdelkhalek et al and Ding et al showed negative results for optrA and poxtA genes in LRCoNS isolates [35, 57].

Future usage of oxazolidinones is expected to have an impact on the resistance distribution, but it is difficult to anticipate which resistance mechanism will predominate [58].

Conclusion

In this study, three LRCoNS isolates (2 *S. epidermidis* and one *S. haemolyticus*) were detected in ICU patients in a university hospital in Egypt. These strains carried the cfr resistance gene which is usually associated with resistance to other antimicrobial classes. These findings could demonstrate an alarming rise in the antimicrobial resistance of the last resort antibiotics in hospital settings. Therefore, a precise antibiotic stewardship program should be applied in hospitals for the proper use of antimicrobial agents.

Limitations

Because of restricted resources, our study has some limitations. In this study, we only detected 3 strains of LRCoNS which make limitations in the statistical analysis of data. Further studies can demonstrate other genes associated with linezolid

resistance such as mutations in 23S rRNA and ribosomal proteins [59]. Also, we identified the CoNS strains in ICU patients, multi-centric further studies can be done for broad results that include community-acquired and hospital-acquired infections.

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Conflict of interest

The authors declare no conflict of interest.

Authors' contribution

Hanaa I. Abd El-Hady: Research concept, study design, Microbiology lab. work and PCR.

Aya M. Abbass, Mahmoud Amer, Ehab Sh. Abdallah: Patient selection, specimens collection and data interpretation

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All authors had full access to all data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

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