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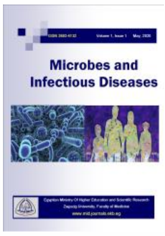

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Original article

Identification and antibiotic susceptibility patterns of multidrug resistant bacteria causing surgical site infections in Suez Canal University Hospitals in Egypt

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ABSTRACT

Background: Surgical Site infections (SSIs) are a major postoperative complication, impacting patients and healthcare systems on a global scale. The rise and prevalence of multidrug-resistant bacteria (MDR) play a significant role in developing SSIs that pose a significant challenge. The aim of the study was to identify the types and antibiotic susceptibility pattern of MDR bacteria causing SSIs in Suez Canal University Hospitals (SCUHs). **Methods:** A descriptive cross-sectional study included eighty wound swabs were collected from patients underwent surgical procedures and suspected to have SSIs. Bacterial growth was identified by conventional methods such as Gram staining, culture on suitable media, and biochemical reactions. Antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion and broth microdilution minimal inhibitory concentration (MIC) methods. **Results:** The prevalence of MDR in SCUHs was 68.9%; 37.8% were MDR, and 31.1% were extensively drug-resistant (XDR). MDR/XDR isolates were 51.6% Gram-positive and 48.4% Gram-negative. Most of the MDR isolates were *S. aureus* (64.7%), and most of the XDR isolates were *Klebsiella pneumoniae* (42.8%). Gram-positive isolates were most resistant to cefoxitin (100%), followed by gentamicin and tetracycline (92.9%) and were most sensitive to vancomycin (100%), levofloxacin (85.7%), and trimethoprim-sulfamethoxazole and chloramphenicol (76.9%). Gram-negative isolates were most resistant to cefoxitin, trimethoprim-sulfamethoxazole, and cephalosporins (100%), followed by meropenem (92.9%) and aztreonam (92.3%), and were most sensitive to chloramphenicol (81.8%), followed by gentamicin (35.7%). **Conclusions:** Multidrug-resistant bacteria represent a considerable health problem at SCUHs. Vancomycin, levofloxacin, and gentamicin can be good choices as empirical treatments for MDR bacteria in SSI infections.

Introduction

Surgical site infections (SSIs) refer to infections that manifest in the incision or organ/space after a surgical procedure. The Centers for Disease Control and Prevention (CDC) defines SSIs as infections that develop at the site of a surgical procedure, generally 30 to 90 days after the surgery, depending on the procedure. SSIs are

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categorized as superficial incisional SSIs, deep incisional SSIs and organ/space. SSIs Superficial incisional SSIs account for over half of all SSIs across various categories of surgery [1].

SSIs are a dangerous complication causing higher healthcare costs and increased illness, leading to longer hospital stays of 7 to 11 days after surgery. Moreover, patients with SSI are at a significantly greater risk of death (2 to 11 times higher than those without SSI after surgical procedures) [2].

SSIs emerge in roughly 2-5% of surgical patients across the globe, with a greater number of patients in developing countries being impacted compared to their counterparts in developed countries. In developing countries, SSI is the major infection affecting more than 60% of the operated patients [3]. Surgical site infections are the most predominant type of healthcare-associated infections (HAIs), accounting for approximately 14-25% of all HAIs [4].

Staphylococcus aureus (S. aureus) is the primary causative agent of SSIs due to its high prevalence as a colonizing bacterium and its virulent pathogenicity [5]. Among the Gram-negative bacterial pathogens commonly linked with SSIs, *Klebsiella* species, *Escherichia coli (E. coli)*, *Acinetobacter* species, and *Pseudomonas aeruginosa (P. aeruginosa)* are frequently encountered [6].

In recent years, there has been an increase in the rates of SSIs, which may be associated with the high prevalence of multi-drug-resistant (MDR) bacteria responsible for these infections [7]. Multidrug-resistant is defined as being resistant to at least one agent in three or more antimicrobial classes. Extensively drug-resistant (XDR) is characterized by non-sensitivity to at least one agent in all but two or fewer antimicrobial groups, meaning bacterial isolates are only susceptible to one or two categories. Pan-drug-resistant (PDR) is described as resistant to all medicines in all antimicrobial classifications [8]. In Egypt, it was found that the rate of SSIs caused by MDR bacteria was up to 79% [7].

Antimicrobial resistance (AMR) is posing high public health concerns at the global level by decreasing the outcomes of antibacterial treatment, increasing morbidity and mortality, elevating the cost of treatment, and creating a high burden on the health care system. Excessive utilization of antimicrobial drugs, dispensing of medications without susceptibility testing, self-treatment practices, and extended hospital stay are contributing factors for the emergence of MDR infections [9]. The aim of this study was to identify the types and antibiotic susceptibility pattern of MDR bacteria causing SSIs in SCUHs.

Materials and methods

Study design:

A descriptive cross-sectional study was carried out in SCUHs, Ismailia, Egypt, from January 2023 to May 2023. This study was approved by the Ethics Committee of the Faculty of Medicine, Suez Canal University.

Study population and setting:

Wound swabs were collected from 80 patients underwent surgical procedures and suspected to have SSIs. Both sexes and all age groups were included. Written informed consent was obtained from study participants.

Identification of bacterial growth

Specimens were cultured on blood agar and MacConkey agar (Oxoid, UK), and incubated aerobically at 37°C for 24-48 hours. Bacterial growth was identified by colony morphology, Gram staining, and biochemical reactions. Gram-positive cocci were identified by catalase test, coagulase test, and mannitol fermentation test. Gram-negative bacilli were identified by indole production test, citrate utilization test, Voges-Proskauer test, lysine decarboxylation test, ornithine decarboxylation test, triple sugar iron test, and sugar fermentation tests [10].

Antibiotic susceptibility testing

Antibiotic susceptibility of the bacterial isolates was done by the Kirbey-Bauer method using Müller-Hinton agar (Oxoid, UK) according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [11]. Vancomycin resistance in *Staphylococcal* isolates was tested by vancomycin screening test and confirmed by vancomycin MIC testing according to CLSI, 2023 [11]. Inducible clindamycin resistance was detected by the double disk diffusion test (D-test) for clindamycin-sensitive and erythromycin-resistant isolates according to CLSI 2023 [11]. Colistin susceptibility was tested by the broth microdilution MIC method according to CLSI, 2018 [12].

Bacteria were identified as MDR if they were resistant to one agent in three or more antimicrobial classes, as XDR if they were resistant to all but two or fewer antimicrobial classes and as PDR if they were resistant to all agents in all antimicrobial classes [7].

Data management

The data were collected and presented in tables. Qualitative data were represented as frequencies and percentages. Results were interpreted and analyzed via the Microsoft Excel 365 program.

Results

From the collected 80 wound swabs, only 45 specimens were culture-positive; 44 specimens (55%) showed bacterial growth and one specimen (1.25%) showed yeast growth. The prevalence of SSIs in SCUHs was 56.3%.

Gram-positive bacteria were the most commonly isolated microorganisms (60%) followed by the Gram-negative (37.8%). Only one yeast isolate was isolated (2.2%). *Staphylococcus aureus* was the most commonly isolated species (48.89%), followed by *K. pneumoniae* (15.56%), then coagulase-negative *Staphylococci* (CoNS) (11.11%). *Enterobacter* species were the least isolated microorganisms (2.22%) **(Table 1)**.

Antimicrobial susceptibility testing (AST) of Gram-positive isolates showed that all the Gram-positive isolates were sensitive to vancomycin (100%) followed by levofloxacin (92.6%), and all isolates were resistant to cefoxitin (all were considered as MRSA) **(Table 2)**. Only two of the 22 *S. aureus* isolates (9.1%) showed inducible clindamycin resistance by (D-test).

Antibiotic susceptibility testing of *Enterobacteriaceae* isolates showed that the majority of isolates (92.3%) were resistant to cefepime, cefoxitin, and ceftazidime, followed by aztreonam, ceftriaxone, meropenem, and trimethoprim-sulfamethoxazole (84.6%). Most of the isolates (76.9%) were sensitive to chloramphenicol **(Table 3)**.

AST of the two *P. aeruginosa* isolates showed that both of them were resistant to cefepime (100%), while aztreonam, ceftazidime, imipenem, levofloxacin and meropenem showed 50% resistance. The XDR strain was sensitive to colistin (MIC = 2 μg/ml). The two *A. baumannii* strains showed 100% resistance to amikacin, cefepime, ceftazidime, ceftriaxone, gentamicin, imipenem, levofloxacin, meropenem and trimethoprim-sulfamethoxazole. Both of them were sensitive only to doxycycline.

Among the 45 culture-positive specimens, 31 isolates showed multiple antimicrobial resistance. The prevalence of multiple antimicrobial resistance among the collected specimens was 68.9%. Seventeen isolates (37.8%) were MDR and 14 (31.1%) were XDR. No pan-resistant strains were isolated. MDR/XDR isolates were 51.6% Gram-positive and 48.4% Gram-negative. Most of the MDR were *S. aureus* (64.7%) followed by CoNS (17.6%), and most of the XDR isolates were *K. pneumoniae* (42.86%) followed by *K. oxytoca* (21.43%) **(Table 4).**

Gram-positive isolates were most resistant to cefoxitin (100%), followed by gentamicin and tetracycline (92.9%) and were most sensitive to vancomycin (100%), followed by levofloxacin (85.7%), then trimethoprim-sulfamethoxazole and chloramphenicol (76.9%). Gram-negative isolates were most resistant to cefoxitin, trimethoprim-sulfamethoxazole, and cephalosporins (ceftazidime, ceftriaxone, and cefepime) (100%), followed by meropenem (92.9%) and aztreonam (92.3%), and were most sensitive to chloramphenicol (81.8%), followed by gentamicin (35.7%) **(Table 5).**

In the MDR/XDR isolates, 25.8%, 12.9%, and 16.1% of them were resistant to 3, 4, and 5 antibiotic groups respectively, while 45.2% of them were resistant to more than 5 antibiotic groups **(Table 6).**

Table 1. Types of isolated microorganisms.

Microorganism	**Number**	**Percentage**
Gram-positive bacteria		
Staphylococcus aureus	22	48.89%
CoNS	5	11.11%
Gram-negative bacteria		
Klebsiella pneumoniae	7	15.56%
Klebsiella oxytoca	3	6.68%
Acinetobacter baumannii	2	4.44%
Pseudomonas aeruginosa	2	4.44%
E. coli	2	4.44%
Enterobacter	1	2.22%
Fungi (yeast)		
Candida species	1	2.22%
Total	45	100%

Table 2. Antibiotic susceptibility pattern of Gram-positive isolates (n=27).

Antibiotic	**Resistant**		**Intermediate**		**Sensitive**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Cefoxitin	27	100%	0	0 %	0	0 %
Erythromycin	8	29.6%	4	14.8%	15	55.6%
Clindamycin	6	22.2%	0	0%	21	77.8%
Gentamicin	18	66.7%	0	0%	9	33.3%
Levofloxacin	2	7.4%	0	0%	25	92.6%
Tetracycline	13	48.1%	1	3.8%	13	48.1%
Trimethoprim-Sulfamethoxazole	3	11.1%	1	3.7%	23	85.2%
Chloramphenicol	3	11.1%	1	3.7%	23	85.2%
Vancomycin	0	0%	0	0%	27	100%

Table 3. Antibiotic susceptibility pattern of the *Enterobacteriaceae* isolates (n=13).

Antibiotic	**Resistant**		**Intermediate**		**Sensitive**	
	No.	**%**	**No.**	**%**	**No.**	**%**
Cefoxitin	12	92.3%	0	0%	1	7.7%
Ceftriaxone	11	84.6%	0	0%	2	15.4%
Ceftazidime	12	92.3%	0	0%	1	7.7%
Cefepime	12	92.3%	0	0%	1	7.7%
Aztreonam	11	84.6%	0	0%	2	15.4%
Imipenem	8	61.5%	2	15.4%	3	23.1%
Meropenem	11	84.6%	1	7.7%	1	7.7%
Levofloxacin	10	76.9%	0	0%	3	23.1%
Gentamicin	7	53.8%	0	0%	6	46.2%
Amikacin	10	76.9%	0	0 %	3	23.1%
Trimethoprim-Sulfamethoxazole	11	84.6%	0	0%	2	15.4%
Chloramphenicol	2	15.4%	1	7.7%	10	76.9%

Table 4. Frequency distribution of detected MDR/XDR isolates.

Microorganism	**MDR**		**XDR**		**Total**		
	No.	**%**	**No.**	**%**	**No.**	**%**	**+ve / -ve**
S. aureus	11	64.7%	1	7.1%	12	38.7%	**Gram +ve** 16 (51.6%)
CoNS	3	17.6%	1	7.1%	4	12.9%	
K. pneumoniae	1	5.9%	6	42.9%	7	22.6%	**Gram -ve** 15 (48.4%)
K. oxytoca	-	-	3	21.4%	3	9.7%	
E. coli	1	5.9%	-	-	1	3.2%	
Enterobacter	1	5.9%	-	-	1	3.2%	
P. aeruginosa	-	-	1	7.1%	1	3.2%	
A. baumannii	-	-	2	14.3%	2	6.4%	
Total	17		14		31		

Table 5. Antibiotic resistance pattern of MDR/XDR isolates according to Gram-reaction.

Antibiotics	**Gram +ve**				**Gram -ve**			
	Resistant		**Sensitive**		**Resistant**		**Sensitive**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Amikacin	-	-	-	-	13	86.7%	2	13.3%
Cefoxitin	16	100%	-	-	12	100%	-	-
Chloramphenicol	3	23.1%	10	76.9%	2	18.2%	9	81.8%
Gentamicin	13	92.9%	1	7.1%	9	64.3%	5	35.7%
Erythromycin	6	50%	6	50%	-	-	-	-
Clindamycin	6	42.9%	8	57.1%	-	-	-	-
Levofloxacin	2	14.3%	12	85.7%	13	86.7%	2	13.3%
Tetracycline	13	92.9%	1	7.1%	-	-	-	-
Trimethoprim-Sulfamethoxazole	3	23.1%	10	76.9%	14	100%	-	-
Aztreonam	-	-	-	-	12	92.3%	1	7.7%
Ceftazidime	-	-	-	-	15	100%	-	-
Ceftriaxone	-	-	-	-	14	100%	-	-
Cefepime	-	-	-	-	15	100%	-	-
Imipenem	-	-	-	-	11	84.6%	2	15.4%
Meropenem	-	-	-	-	13	92.9%	1	7.1%
Vancomycin	-	-	16	100%	-	-	-	-

Table 6. Number of antibiotic groups to which the MDR/XDR isolates showed resistance.

No. of antibiotic groups	**Number**	**Percentage**
3 antibiotic groups	8	25.8%
4 antibiotic groups	4	12.9%
5 antibiotic groups	5	16.1%
More than 5 antibiotic groups	14	45.2%
Total	31	100%

Discussion

In this study, the prevalence of SSIs in SCUHs was 56.3%. This was lower than that of **Zahran *et al.***[13] study in Menoufia, in which they found the prevalence of SSIs was 67.6%. Also, **Abosse *et al.***[14] found the overall prevalence of culture-confirmed surgical wound infection was 69.7%. Differences in the prevalence rates among different areas are due to variations in the application of infection control strategies and antibiotic policies.

From the collected 80 wound swabs, only 45 specimens (55%) showed bacterial growth, one specimen (1.25%) showed *Candida* species, while the other 43.75% of the specimens showed no growth. This greatly differs from the study of **Ali *et al.***[15] in which 83.7% of their specimens showed bacterial growth. Also, **Alkaaki *et al.***[16], found that only 23% of cultured bacteria were sensitive to the prophylactic antibiotic given preoperatively. The negative culture specimens in this study might be due to the use of appropriate empirical antibiotics pre- and post-operatively.

Only one strain (1.25%) of *Candida* species was isolated. **Bekiari *et al.***[17] identified 8.4% of their isolates as *Candida* species, while **Shah *et al.***[18] found only 4%. The study of **Jarvis** [19] informed that the ratio of fungi, especially *C. albicans*, is increasing considerably in SSIs. The improper use of chemotherapeutic agents for longer periods as prophylactic drugs alters the microflora of patients which may increase the risk of *Candida* infection in surgical patients **Azevedo *et al.***[20].

Gram-positive bacteria were the most commonly isolated microorganisms (60%) followed by Gram-negative (37.8%). *Staphylococcus aureus* was the most commonly isolated species (48.89%). Similarly, **Chaudhary *et al.***[21] found that *S. aureus* is the most predominant isolate accounting for 47.4% of their specimens. Also, in the study of **Roumbelaki *et al.***[22], they found Gram-positive microorganisms accounted for 52.1% of SSI isolates, however, they found that *Enterococci* were predominant.

Antimicrobial susceptibility patterns of Gram-positive isolates showed that all these isolates were sensitive to vancomycin (100%) and levofloxacin (92.6%), and all of them were resistant to cefoxitin and hence identified as methicillin-resistant *S. aureus* (MRSA). The study of **Khorvash *et al.***[23] confirmed that 78.9% of their isolates were *S. aureus*, and all of them were MRSA. Two *S. aureus* isolates (9.1%) showed inducible clindamycin resistance by D-test. This is nearly equal to the study of **Yehouenou *et al.***[24] in which 9.3% of their *S. aureus* isolates showed inducible clindamycin resistance.

Antimicrobial susceptibility of *Enterobacteriaceae* isolates showed that the majority of the isolates (92.3%) were resistant to cefepime, cefoxitin, and ceftazidime, followed by aztreonam, ceftriaxone, meropenem, and trimethoprim-sulfamethoxazole (84.6%). Most of the isolates (76.9%) were sensitive to chloramphenicol. Similarly, the study of **Yehouenou *et al.***[24] revealed that Gram-negative bacilli show high resistance to ceftazidime, ceftriaxone, and cefepime.

The study included two strains of *P. aeruginosa* and two strains of *A. baumannii.* Antimicrobial susceptibility of the two *P. aeruginosa* isolates showed that both of them were resistant to cefepime (100%). One strain of them was also resistant to aztreonam, ceftazidime, imipenem, levofloxacin, and meropenem. It was tested for colistin susceptibility and it was sensitive to it (MIC = 2 µg/L). This differs from the study of **Khorvash *et al.***[23] who registered 16.7% and 8.3% resistance of *P. aeruginosa* to imipenem and meropenem respectively. On the other hand, it agrees with the study of **Alikhani *et al.***[25] who found all their *P. aeruginosa* showed no sensitivity to cefepime.

The two strains of *A. baumannii* (100%) showed resistance to all the tested antibiotics except doxycycline. **Manyahi *et al.***[26] found the prevalence of MDR for *A. baumannii* was 100%, while **Bediako-Bowan *et al.***[27] found it 52%.

The two *A. baumannii* strains were sensitive only to doxycycline. **Ifa *et al.***[28] found only 28.3% of their isolates sensitive to doxycycline. **Falagas *et al.***[29] stated that treatment of carbapenem-resistant *A. baumannii* with doxycycline-based therapy presents a high clinical success rate reaching up to 76%.

This study showed that the prevalence of MDR among the collected specimens was 68.9%. **Viehman *et al.***[30] identified 53% of their isolates as MDR. Surprisingly, the results of **Hagihara *et al.***[31] revealed only 7.5% of the post-operative infections were due to antimicrobial-resistant bacteria. The current study also showed that 37.8%

of the isolates were MDR and 31.1% were XDR. Most of them (51.6%) were Gram-positive and 48.4% were Gram-negative. Different results were reported in the study of **Raouf *et al.***[32] in which MDR was detected in 13% of isolates, 54.3% were XDR and 10.9% were PDR.

Most of the MDR bacteria were *S. aureus* (64.7%) and most of the XDR isolates were *K. pneumoniae* (42.86%). Differently, *E. coli* was the predominant isolated MDR pathogen (35.8%), followed by *S. aureus* (21.8%) in the study of **Mohamed *et al.***[33]. **Manyahi *et al.***[26] found the overall MDR rate among Gram-positive and Gram-negative bacteria was 60.6% and 61.4%, respectively.

Most (45.2%) of the MDR/XDR isolates were resistant to more than 5 antibiotic groups. In the study of **Manyahi *et al.***[26], the majority (97%) of the Gram-negative bacteria were resistant to more than four classes of antibiotics. Similarly, **Upreti *et al.***[34] found more than 75% of their isolates showed antibiotic resistance to 5 or more antibiotic groups.

The data gathered from this study showed that vancomycin and levofloxacin can be good choices as empirical treatments for Gram-positive MDR infections in SSIs. For Gram-negative bacteria, although most of them were sensitive to chloramphenicol, it should not be recommended as an empirical treatment for these infections because of its undesirable side effects. So, the second choice is recommended; which is gentamicin. The study also emphasized that despite the advancement achieved in surgical techniques, the problem of SSIs, especially with MDR pathogens, remains a pressing concern for healthcare professionals in medical facilities such as SCUHs.

The major limitations of the study were the small sample size and the unavailability of some antimicrobial discs.

Conclusion

MDR bacteria in SSIs represent a considerable health problem at SCUHs. Strict antibiotic policies and infection control measures should be implemented to ensure precise treatment and control of infections caused by these bacteria. Vancomycin, levofloxacin, and gentamicin can be good choices as empirical treatments for MDR bacteria in SSI infections.

Conflicts of interest

There are no conflicts of interest.

Financial disclosures

We declare no financial disclosures.

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