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Original article

Prevalence and antimicrobial resistance patterns of nosocomial pathogens causing surgical site infections in an Egyptian university hospital

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ABSTRACT

Background: Surgical site infections (SSI) are a common type of health care associated infections. The emergence of multidrug resistant (MDR) nosocomial pathogens represents a major health burden. This study was conducted to determine the frequency of isolation and patterns of antimicrobial resistance of nosocomial pathogens causing SSI in Zagazig University Hospitals **Methods:** Samples obtained from the infected surgical wounds were subjected to microbiological identification and antibiotic susceptibility testing. The role of extended spectrum beta lactamase (ESBL) and carbapenemase in bacterial resistance to some antibiotic were evaluated. **Results:** The most frequently isolated species were *S. aureus* (31%) followed by *Klebsiella pneumoniae* (*K. pneumonia)* (22%), *Escherichia coli* (*E. coli)* (15%), *Pseudomonas aeruginosa (P. aeruginosa)* (11%), Coagulase negative staphylococci (CoNs) (8%), *Proteus* spp (7%) and *Acinetobacter* spp (6%). Methicillin resistance was detected in 38 (97 %) and 8(80 %) of *S. aureus* and CoNs isolates, respectively. Among Gram-negative organisms, 65.8% of isolates were ESBL producers, of which 60% were Carbapenem resistant. Metallo-β-lactamase was detected in 30% of Gram-negative isolates. Multi-drug resistance was observed in 50 isolates (68.5%), whereas extensively drug resistance (XDR) occurred in 23(31.5%) of Gram-negative isolates. **Conclusions:** Most of Gram-negative isolates were MDR or XDR. Antibiotic therapy of SSI must be guided by microbiological culture and antibiotic sensitivity testing. Infection prevention and control practice needs more improvement. Rationalization of antibiotic prescription must be carried out. Post discharge surveillance of SSI needs to be considered.

Introduction

Surgical site infections (SSIs) are potential complications associated with any type of surgical procedure; they are one of the leading causes of hospital acquired infection (HAI) in low- and middle-income countries. Strict infection prevention and control measures have the ability to prevent SSI, so, the prevention of SSI has received considerable attention from the infection control professionals and health care authorities [1].

There are many definitions of SSI, it was defined by the European Centers for Disease Control and Prevention as an infection that occurs within 30 days after the operation and involves the skin and subcutaneous tissue at the site of incision (superficial incisional) and/or the deep soft tissues of the incision

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(deep incisional) and/or any part of the anatomy (for example, organs and spaces) other than the incision that was opened or manipulated during an operation (organ/space) [2].

Outcomes of SSIs are of wide spectrum; hence, some cases are just presented with trivial wound discharge, and others may suffer from life threatening conditions with considerable morbidity [3].

Many factors determine the potential for SSI. Some of these factors are patient related; age, sex, diabetes mellitus, obesity, tobacco use and antibiotic use, others are procedure-related; wound class, length of surgery, hypoxia, hypothermia and infection control practice [4].

Rapid emergence and spread of extended spectrum beta lactamases (ESBL) and different carbapenemases (MBL, OXA 48, KPC, AmpC) among bacteria definitely makes choosing an appropriate antimicrobial a difficult issue [5]. The resulting Multidrug resistance (MDR), extensive drug resistance (XDR) and pan drug resistance (PDR) represent a great challenge that require strict adherence to infection control precautions and rational antibiotic use [6]. It was reported that 54.1% of *Enterobacteriaceae* isolated from Egyptian intensive care units (ICUs) were carbapenem resistant [7]

Therefore, identification of microbes and determining their antibiotic susceptibility patterns will assist in selection of the convenient chemotherapy and decrease the chance of resistance in bacterial community.

This study was conducted to identify the frequency of isolation and patterns of antimicrobial resistance of nosocomial pathogens causing SSI in Zagazig University Hospitals (ZUH).

Methods

This cross-sectional study was carried out from April 2019 to January 2020 in Microbiology and Immunology department, 135 patients visiting the hospital outpatient clinics for wound care and follow up were enrolled. The inclusion criteria included: patients that underwent surgical procedures in ZUH within 30 days, and clinically suspected to have SSI; fever, tenderness, pain, or discharge from the surgical site, and their surgical wound exclusively cared and dressed in the ZUH. Patients that had surgical interventions, or any wound dressing outside the ZUH were excluded.

Demographic data (age, sex, social status and residence, etc.), clinical data (ICU admission, devices, antibiotic administration) were collected from all patients. Medical history of comorbidities; diabetes, obesity, etc. and surgical history; length of the procedure, type of the wound (clean, clean contaminated, contaminated, or dirty), prosthesis or implant insertion and length of postoperative stay were reported for each patient.

Sample collection

The wound was exposed under complete aseptic conditions, after removing excess secretions, it was cleansed using sterile normal saline and samples were aspirated by sterile syringes if possible or by rotating sterile culture swabs with sufficient pressure. Written consents were taken from all patients. This study was approved by Zagazig University Institutional Review Board.

Microbiological processing of samples

- ***Cultivation***

Gram films were performed and examined to assess the presence of pus cells or microorganisms. Cultivation was performed on Blood agar, MacConkey, and Nutrient agar (Oxoid), all plates were incubated aerobically at 35-37^{o}C for 24 hours and then examined for bacterial growth. Syringes from deep wounds were additionally incubated anaerobic. The samples were processed within 2 hours, when delay was inevitable Amies transport medium was used [8]

- ***Identification***

Identification was done by colony morphology, Gram staining and biochemical reactions; **(Flowchart below)**. Catalase, Coagulase and DNAse tests were performed for Gram positive cocci isolates. Gram negative isolates were identified using oxidase, triple sugar iron, citrate utilization, urease, indole and motility tests **(Table 1)**. API 20E strips were used for further confirmation of some *Enterobacteriacae* isolates. Identification of *P. aeruginosa* isolates was confirmed by their strict aerobic property, the characteristic odor and exopigments and their ability to grow at 42^{o}C. *Acinetobacter* spp were suspected by their Gram film appearance; plump Gram-negative or Gram variable rods/coccobacilli or cocci. Colonies could be encapsulated, mucoid or hemolytics. oxidase negative and can grow anaerobic. In case of negative culture results, other samples were obtained and processed [8].

- ***Antimicrobial testing***

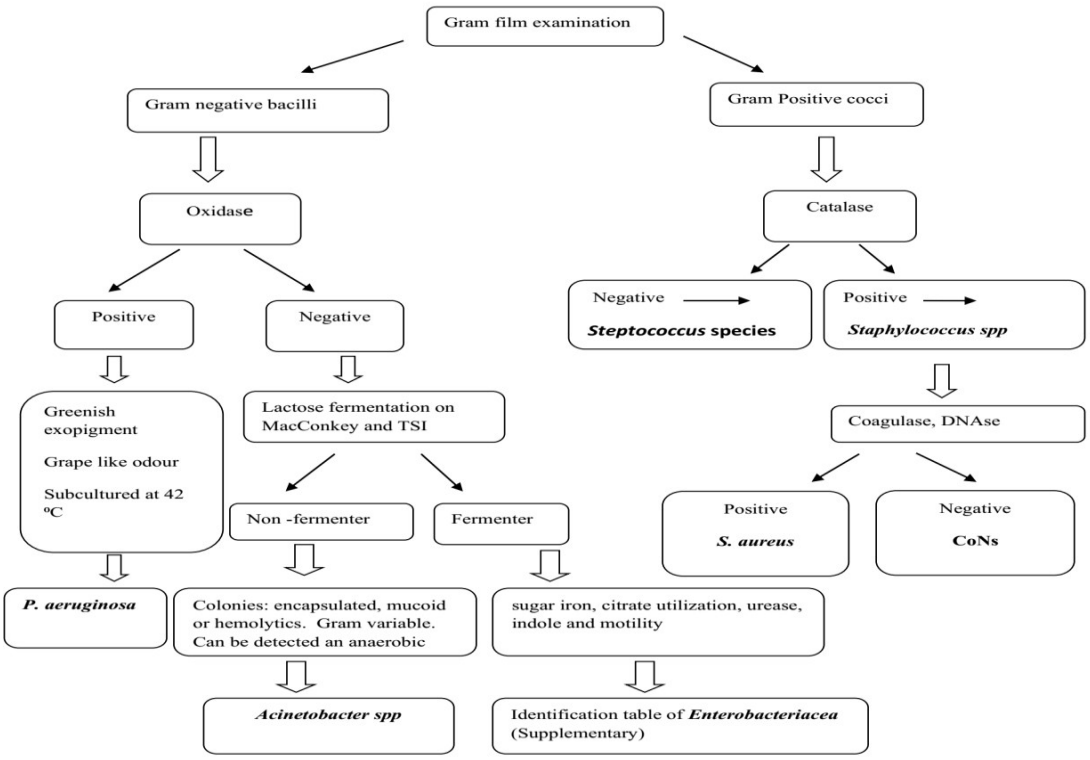
Antibiotic susceptibility tests were performed for the isolated pathogenic strains by modified Kirby-Bauer's disc diffusion technique on Muller Hinton agar according to Clinical and laboratory standards institute (CLSI) 2020 guidelines [9]. *Staphylococcus aureus (S.aureus)* isolates with cefoxitin zone diameter of 21 mm or less were reported as oxacillin (methicillin) resistant, Coagulase negative staphylococci (CoNs) isolates with cefoxitin zone diameter of 24 mm or less and oxacillin zone diameter of 17 mm or less were reported as oxacillin (methicillin) resistant, as per the CLSI updates that recommend oxacillin testing to detect methicillin resistance is some species of coagulase negative staphylococci [9]. Gram negative isolates that exhibited resistance for agents of third generation cephalosporins (ceftazidime,cefotaxime, ceftriaxone) were subjected to combined disc diffusion test to confirm ESBL production, enhancement of the inhibition zones around cefotaxime and ceftriaxone discs toward amoxicillin-clavulanic disc indicating that strain had ESBL activity [9]. Gram-negative isolates that exhibited resistance to imipenem or meropenem were further tested by MASTDISCS® Combi Carba plus, this kit allows detection of four carbapenemases; Metallo Beta lactamase (MBL) , KPC, AmpC and OXA-48 .It consists of 5 discs; (A) Carbapenem 10ug, (B) Carbapenem 10ug + MBL inhibitor, (C) Carbapenem 10ug + KPC inhibitor, (D) Carbapenem 10ug + AmpC inhibitor and (E) Temocillin + MBL inhibitor . If a difference of 5 mm or more in disc zone diameters was only observed between Disc A and B, this indicates MBL production. Similarly, more than 5 mm difference only between Disc A and Disc C indicates KPC production.

When both Discs C and D show zone differences of 5mm or more compared with Disc A, AmpC production is indicated. OXA-48 production is indicated when an inhibition zone diameter of 10mm or less around Disc E as the high-level resistance to Temocillin is characteristic of OXA-48 [10]. Quality control of the culture media, Gram stain, and antimicrobial sensitivity tests was checked using standardized reference strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923) and *P. aeruginosa* ATCC 27853 [9].

Multidrug resistant (MDR) bacteria were considered if the bacterial isolate was non-susceptible to at least one agent in three or more antimicrobial categories, The isolate was considered XDR if it was non-susceptible to at least one agent in all but two or fewer antimicrobial categories, Pan drug resistant (PDR) was defined as non-susceptible to all agents in all antimicrobial categories [6].

Statistical analysis was performed using SPSS software version16.0

Flowchart of the identification process [8]



Results

The mean age of the study participant was 42±13 years, 84 males and 51 females. 58% of subjects were diabetics, 55% of them were obese. About 95% of subjects received prophylactic antibiotics. Urgent surgical procedures were performed for 72% of them. The wound class of the majority of participants was contaminated and clean/contaminated (46%, 32% respectively), 14% of subjects had clean wounds and 8% of them had infected wounds. More than half of study population had postoperative stay period less than 1 week **(Table 1)**.

Bacterial growth was detected in about 90% of samples (n=122). Microbiological profile of isolated micro-organism is displayed in **table (2)**. The most frequently isolated species were *S. aureus* (31%) followed by *Klebsiella pneumoniae* (*K. pneumonia*) (22%), *E. coli* (15%), *P. aeruginosa* (11%), CoNs (8%), *Proteus* spp (7%) and *Acinetobacter* spp (6%). *Candida* spp was isolated from five samples in addition to the bacterial pathogens.

Staphylococcus aureus exhibited absolute resistance for amoxicillin/clavulanic acid ampicillin/sulbactam. Resistance rates of *S. aureus* for gentamycin and trimethoprim /sulfamethoxazole were 90% and 97% respectively. Methicillin resistance was detected in 97% of *S. aureus* (based on cefoxitin resistance). Agents with relatively higher sensitivity rate were; linezolid, teicoplanin, rifampicin and imipenem **(Table 3)**.

Resistance for amoxicillin/clavulanic acid ampicillin/sulbactam was detected in 100% of CoNs isolates, Methicillin resistance was detected in 80% of CoNs isolates (based on results of oxacillin and cefoxitin). Resistance rates for the other tested agents were; 70% for trimethoprim /sulfamethoxazole, 60% for each of gentamycin and teicoplanin, 50% for each of ciprofloxacin and imipenem, 40% for linezolid and 30% for rifampicin **(Table 3)**.

Isolates of *K. pneumonia, P. aeruginosa and Acinetobacter* spp exhibited 100% resistance for ampicillin-sulbactam, amoxicillin+ clavulanic acid, cefoxitin and cefotaxime. *Klebsiella pneumonia* isolates showed high resistance rates for ceftazidime (93%), piperacillin /tazobactam (89%), cefuroxime (85%), and (81%) **(Table 4).**

Escherichia coli showed high resistance rates for cephalosporin agents (67-94%) and absolute resistance for piperacillin /tazobactam. Resistance rates for trimethoprim /sulfamethoxazole, ampicillin-sulbactam and amoxicillin-clavulanic acid, were 78, 56, 44% respectively. Aminoglycoside agents and levofloxacin showed relatively low resistance rates. The lowest resistance rates observed for meropenem and Imipenem (6, 17%), respectively. **(Table 4)**.

Pseudomonas aeruginosa showed high resistance rates for most tested antibiotic agents except for imipenem and amikacin. *Acinetobacter* spp showed absolute resistance for cefuroxime, trimethoprim /sulfamethoxazole and meropenem, resistance rates for imipenem and cefepime was 86% for each **(Table 4)**.

The overall antimicrobial susceptibility patterns of Gram-negative isolates are displayed in **table (5)**, imipenem and meropenem owned the highest sensitivity rates (68.5, 58.9) % respectively, followed by gentamycin (56.2%), levofloxacin (54.8%) and amikacin (40%). Generally, there were remarkable levels of resistance for the tested cephalosporin agents. 68.5% of Gram-negative isolates (n: 50) were MDR while 31.5% were XDR (n: 23). No pan drug resistance pattern could be detected. ESBL production was confirmed using combined disk-diffusion test in 65.8% (n=48) of Gram-negative isolates.

Carbapenem resistance (imipenem, meropenem, or both) was detected by disc diffusion methods in about 60% (n=43) of Gram-negative isolates. The majority of these isolates were *K. pneumonia* (44.2%) and *P. aeruginosa* (30.2%). Carbapenem resistance was detected in all isolates of *Acinetobacter spp* and *P. aeruginosa* and in (70% and 22%) of *K. pneumonia* and *E. coli* isolates respectively. All isolates of *Proteus* spp were carbapenem sensitive **(Table 6)**.

Metallo-β-lactamase (MBL) was detected in 30% (n=22) of Gram-negative isolates, with a tendency to appear more in Non-*Enterobacteriaceae* than *Enterobacteriaceae,* this difference was statistically significant. On the other hand, Oxa -48 was detected in 17.8 % (n:13) of Gram-negative bacteria, there was no statistically significant difference for Oxa- 48 between *Enterobacteriaceae* and non-*Enterobacteriaceae.* Neither KPC nor AmpC could be detected among the tested isolates **(Table 6,7)**.

Table 1. Demographic and clinical criteria of the study participants.

		Frequency	Percentage
Age	42±13 (17-62)		
Gender	Male	84	62
	Female	51	38
Department	Surgery	93	69
	Orthopedics	42	31
Diabetes	Yes	78	58
	No	57	42
Obesity	Yes	75	55
	No	60	45
ICU admission	Yes	34	25
	No	101	75
Condition of procedures	Urgent	97	72
	Elective	38	28
Class of wound	Clean	19	14
	Clean contaminated	43	32
	Contaminated	62	46
	Infected	11	8
Prophylactic antibiotics	Yes	128	95
	No	7	5
Post-operative stay period	Less than 1 week	76	56
	1-2 weeks	42	31
	More than 2 weeks	17	13

Table 2. Microbiological profile of the isolated micro-organisms.

Organism	**Total = 122**	
	No	**%**
S. aureus	39	31
Klebsiella pneumonia	27	22
E. coli	18	15
P. aeruginosa	13	11
CONs	10	8
Proteus	8	7
Acinetobacter	7	6

Table 3. Antibiotic Sensitivity patterns of Gram-positive isolates.

Oxoid antibiotic disc	**Conc.**	***S. aureus* (n:39)**				**CoNs (n:10)**			
		S		**R**		**S**		**R**	
		No	**%**	**No**	**%**	**No**	**%**	**No**	**%**
AMC	20/10	0	0	39	100	0	0	10	100
SAM	10/16	0	0	39	100	0	0	10	100
Oxacillin	1	1	3	38	97	2	20	8	80
Cefoxitin	30	1	3	38	97	2	20	8	80
Gentamycin	10	5	10	35	90	4	40	6	60
Ciprofloxacin	5	7	8	32	82	5	50	5	50
Imipenem	10	25	64	14	36	5	50	5	50
Linezolid	30	33	85	6	15	6	60	4	40
Teicoplanin	30	33	85	6	15	4	40	6	60
Rifampicin	5	26	67	13	33	7	70	3	30
SXT	1.25/23.75	1	3	38	97	3	30	7	70

Ampicillin-sulbactam (SAM), Amoxicillin-clavulanic acid (AMC), Trimethoprim /Sulfamethoxazole (SXT).

Table 4. Antibiotic Sensitivity patterns of Gram-negative isolates (%).

Oxoid antibiotic discs	*K. pneumonia* N: 27		*E. coli* N: 18		*P. areuginosa* N: 13		*Acinetobacter* spp N: 7		*Proteus* spp N: 8	
	S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
AMC (20/10 ug)	0	100	56	44	0	100	0	100	86	14
SAM (10/16 ug)	0	100	44	56	0	100	0	100	38	62
Cefotaxime (30 ug)	0	100	33	67	0	100	0	100	62	38
Ceftriaxone (30 ug)	11	89	22	78	23	77	0	100	12	88
Cefepime (30 ug)	15	85	6	94	30	70	14	86	12	88
Cefoxitin (30 ug)	0	100	6	94	0	100	0	100	37	63
Ceftazidime (30 ug)	7	93	22	78	0	100	28	72	50	50
Cefuroxime (30 ug)	15	85	6	94	0	100	0	100	25	75
Meropenem (10 ug)	56	44	94	6	23	77	0	100	100	0
Imipenem (10 ug)	74	26	83	17	46	54	14	86	100	0
Amikacin (30 ug)	22	78	56	44	70	30	28	72	25	75
Gentamycin (10 ug)	63	37	61	39	46	54	28	72	62	38
Levofloxacin (5 ug)	33	67	72	28	23	77	28	72	75	25
Piperacillin /Tazobactam (100/10 ug)	11	89	0	100	23	77	28	72	25	75
Trimethoprim /Sulfamethoxazole (1.25/23.75 ug).	19	81	22	78	0	100	0	100	0	100

Ampicillin-sulbactam (SAM), Amoxicillin-clavulanic acid (AMC), Trimethoprim /Sulfamethoxazole (SXT).

Table 5. Antimicrobial susceptibility patterns of all Gram-negative bacteria isolates (n=73).

	Susceptible		Resistant	
	No	**%**	**No**	**%**
AMC (10/16 ug)	17	23	66	87
SAM (20/10 ug)	11	15	62	85
Cefotaxime (30 ug)	12	16	61	84
Ceftriaxone (30 ug)	10	13.6	63	86.4
Cefepime (30 ug)	10	13.6	66	86.4
Cefoxitin (30 ug)	4	5.5	66	94.5
Ceftazidime (30 ug)	10	13.6	63	86.4
Cefuroxime (30 ug)	6	4.2	70	95.8
Meropenem (10 ug)	43	58.9	30	41.1
Imipenem (10 ug)	50	68.5	23	31.5
Amikacin (30 ug)	29	40	44	60
Gentamycin (10 ug)	41	56.2	32	43.8
Levofloxacin (5 ug)	63	45.2	40	54.8
Piperacillin /Tazobactam (100/10 ug)	9	12.3	64	87.7
Trimethoprim/Sulfamethoxazole (1.25/23.75 ug)	6	8.2	67	91.8

Ampicillin-sulbactam (SAM), Amoxicillin-clavulanic acid (AMC), Trimethoprim/Sulfamethoxazole (SXT).

Table 6. Distribution of carbapenem resistance among various Gram-negative isolates and among each species.

	Carbapenem resistant Gram-negative bacteria (n=43)				
Organism	**No.**	**%**	**Total no. of each species**	**Carbapenem resistance in each species**	
				No.	**%**
K. pneumoniae	19	44.2	27	19	70
P. aeruginosa	13	30.2	13	13	100
***Acinetobacter* spp**	7	16.3	7	7	100
E. coli	4	9.3	18	4	22

Table 7. Occurrence of carbapenem resistance using phenotypic test among *Enterobacteriaceae* and Non–*Enterobacteriaceae.*

	Enterobacteriacea	***Non- Enterobacteriacea***	**Chi**	***P***
MBL positive	8	14	5.3	*0.02**
MBL negative	15	6		
Oxa 48 positive	7	6	0.0	*0.9*
Oxa48 negative	16	14		

**P < 0.05* considered significant

Discussion

Surgical site infections were defined as "infection that occurs at incision site within 30 days after surgery". Infection of surgical wound is a real problem especially in developing countries. The WHO global guidelines for prevention of SSI reported that a significant proportion of SSIs (13-71%) had been detected following patient discharge [1]. The growing intention to decrease hospital length over the last years made post-discharge surveillance recommended by many surveillance networks [11, 12].

It is of a great importance to understand the pattern of isolation and antimicrobial susceptibility to allow data needed to review and evaluate infection control practice in the health care environment [13].

In this study, post discharge follows up was done for patients that underwent surgical procedures in the ZUH within 30 days. We reported the frequency of isolation of pathogens to detect the most prevalent species, susceptibility and resistance rates of various antibiotics were calculated. Percentages of specific resistance patterns (MRSA, ESBL, carbapenem resistance) were estimated.

In the current study, most of subjects had contaminated or clean/contaminated wounds, dirty wounds detected in 7% of patients, this is partially agreed with Zahran et al., their study reported that contaminated wound was the most prevalent wound class of patients with SSI followed by infected wound [14].

In this study, the majority of subjects received antibiotic prophylaxis. More than 70% of the performed procedures were urgent. Out of 135 patients with clinically suspected SSI, 122 patients were culture positive. This could be explained by the administration of antibiotics or the anaerobic bacteria as anaerobic cultures were only done for syringes from deep wounds. The prevalence of culture positive samples was 90%, this is nearly similar to culture positive rate recorded by **Dessie et al.** [15].

We noticed that S. *aureus* was the leading isolated pathogen. This finding is a cardinal feature of SSI as many studies have stated that *S aureus* was the commonest isolate from the wound infections in many countries [13-16] including Egypt [5,14].

Most of S. *aureus* isolates of this study exhibited methicillin resistance (94%), this finding is quite similar to that obtained by **Dessie at al.** [15], other studies reported that isolation rates of MRSA were ranging from 60-88% [14,17,18]. Lower MRSA isolation rate was reported by **Kalayu et al.** [13]. Coagulase negative staphylococci accounted for 8% of the isolated pathogens, however, methicillin resistance pattern was detected in 80% them, this agrees with **Yishak et al.** [19].

Although S.*arueus* was the most frequently isolated single pathogen, we noticed that most isolated organisms were Gram negative bacilli with predominance of *K. pneumonia* followed by *E. coli* and *P. aeruginosa.* These observations come in accordance with other studies [13, 15, 20]. However, *Proteus mirabilis* was the predominant pathogen among the isolated bacteria in a study conducted in Sudan [21]. *Pseudomonas aeruginosa* and *E. coli* were the commonest isolates from wounds in other studies [22, 23]. Bacterial species and prevalence may vary due to the geographical distribution of causative agents, the type of performed surgical procedure and the strength of infection control practice.

Most of Gram-negative bacteria were resistant to penicillins and cephalosporins; an observation that seems to be common in many other

studies [13,15,16], however we noticed that amoxicillin-clavulanic was effective in about half of *E. coli* isolates. This observation emphasizes the role of bacterial culture and sensitivity to guide treatment options of such types of infections.

Among the tested antimicrobial agents, carbapenems and aminoglycoside were the most effective options, this finding is similar to those of **Chaudhary et al.** [16]. Pervious study had indicated that Gram negative uropathogens exhibited excellent sensitivity to meropenem and aztreonam [24]. This study showed that 65.8% of Gram-negative bacteria were confirmed to be ESBL producer by combined disc test, this comes in accordance with some studies [14, 25], lower rates were reported in other studies [24, 26].

In this study, carbapenem resistance (imipenem, meropenem, or both) was detected in about 60% Gram negative isolates, an Egyptian study reported that 54.1% of *Enterobacteriaceae* isolated from Egyptian intensive care units were carbapenem resistant [7].

We observed that the most common carbapenem resistant Gram-ve isolates were *K. pneumonia* and *P. aeruginosa* followed by *E. coli* and *Acinetobacter* spp. This agreed with **Silva et al.** [27] and partially agreed with **Solanki et al.** [28] they found that *K. pneumonia* was the most prevalent carbapenem resistant isolates followed by *E coli*, *Acenitobacter* spp and *P. aeruginosa*. **Hasanin et al.** [29] found that *Acinetobacter* accounted for (86%) of carbapenem resistant isolates, although the relatively small number of carbapenem resistant *Acenitobacter* isolates in our study, this number represent 100% of this bacterial species.

We noticed that Metallo-β-lactamase was detected in 30% of Gram negative bacteria, this rate is lower than that obtained by a similar Egyptian study in which more than 70% of Gram negative isolates were MBL producer, and this difference may be due to different methodology [14]. In the current study, MBL tends to appear more among Non-enterobacteriaceae than *Entrobacteriaceae* isolates, this observation comes in accordance with **Diwakar et al.** [30]. In this research, MDR and XDR Gram negative bacteria isolation rates were 68.5 and 31.5%, respectively. Lower rates were reported in some studies [16, 31]. Other Egyptian studies showed variable isolation rates of MDR ranging from 37.2 to 95%, with observed chronologically increasing rates [14,24, 32].

Conclusion

Staphylococcus aureus was the most prevalent organism isolated from SSI. Most of Gram-negative isolates were MDR or XDR. Antibiotic therapy of SSI must be guided by microbiological culture and antibiotic susceptibility testing. Infection prevention and control practices need more improvement. Rationalization of antibiotic prescription must be carried out. Post discharge surveillance of SSI needs to be applied.

Conflicts of interest: no conflicts of interest.

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Supplementary material

Table I. Identification of *Enterobacteriacea.*

Species	Test/substrate								
	Lac	**Glu**	**Suc**	**mot**	**Gas**	**Ind**	**Cit'**	**Ure**	**H2s**
Echerichia coli	+	+	+	+	+	+	+	-	-
Citrobacter freiindii	+/-	+	+/-	+	+	-	+	+/-	+/-
Klebsiella pneumonia	+	+	+	-	++	-	+	+	-
Klebsiella oxytoca	+	+	+	-	+	-	+	-	-
Enterobacter species	+	+	+/-	+	+	-	+	+/-	-
Serratia marcescens	-	+ -	+	+	+/-	-	+	-	-
Proteus mirabilis	-	+	+/-	+	+	-	+/-	++	+
Proteus vulgaris	-	+	+	+	+	+	-	++	+/-
Pseudomonas	-	-	-	+	+/-	+	+	-	-
Acinetobacter	-	-	-	-	+	+/-	+	_	-

Mot: motility Glu: glucose Ind: Indole product **Cit:** citrate utilization. **H2S:** H2S production (-): >85% of strains negative **Lac:** fermentation of lactose. **Suc:** sucrose. **Ure:** Urease production. **Key:** (+): > 85% of strains positive after 24-48 hours incubation [8].

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