Zagazig J. Pharm. Sci. June, 2018 ISSN 1110-5089
Vol. 27, Issue 1, pp. 31- 38 ISSN (on-line) 2356_9786

Prevalence of multi-drug resistant Staphylococci isolated from surgical site infections

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ABSTRACT

Surgical site infections are the most common post-operative infections even in hospitals with most modern facilities complications and standard protocols of pre-operative preparation and antibiotic prophylaxis.

Staphylococci stay as our natural flora and yet sometimes threaten our life as tenacious pathogens. In addition to their ability to evade our immune system, the multi-drug resistance phenotype makes Staphylococci the most intractable pathogenic bacteria in the history of antibiotic chemotherapy. Staphylococci are among the leading causes of nosocomial infections such as surgical site infections. Increasing resistance to β-lactams and the glycopeptides complicates treatment of infections caused by Stahpylococci. The aim of this study is to investigate the antibiotic resistance profile of Staphylococci isolated from surgical site infection.

Staphylococci isolates were identified morphologically, by Gram stain and biochemical tests. Antimicrobial susceptibility testing was done by the Kirby-Bauer standard disk diffusion method. One hundred Staphylococci isolates were recovered from one hundred and ninety samples isolated from surgical site infections. *Staphylococcus aureus* was the most predominant one. From 100 isolates, *Staphylococcus aureus* was found in 91 isolate and coagulase-negative Staphylococci (CoNS) in 9 isolates. *Staphylococcus aureus* isolates were highly resistant to tigecycline, oxacillin, ampicillin and ampicillin-sulbactam antibiotics. They showed intermediate resistance to daptomycin, amikacin, azithromycin, levofloxacin, clindamycin, sulfamethoxazole-trimethoprime, doxycycline and gatifloxacin, while they showed low resistance to vancomycin, linezolid and imipenem. On the other hand, CoNS isolates were highly resistant to doxycycline, oxacillin, ampicillin and ampicillin- sulbactam antibiotics. They showed intermediate resistance to daptomycin, levofloxacin, clindamycin, sulfamethoxazole-trimethoprime, gatifloxacin, vancomycin, tigecycline, linezolid and imipenem, while they showed low resistance to amikacin and complete sensitivity to azithromycin. Eighty four isolates were multi-drug resistant.

Percentage of multi-drug resistant Staphylococci isolates were very high. This may be attributed to the misuse of antibiotics. To minimize resistance, strict antimicrobial prescription policy should be applied.

Keywords: Staphylococci, Multi-drug resistance, surgical sites

INTRODUCTION

Surgical site infections (SSIs) are defined as infections of skin or underlying soft tissues at the surgical site occurring within 30 days, following National Healthcare Safety Network (NHSN) operative procedure, in which an incision was closed primarily **(CDC, 2013)**. There are three types of SSI; superficial incisional, deep incisional and organ/space SSI **(CDC, 2013).** In clean surgeries methicillin resistant *Staphylococcus aureus* (MRSA) is the most predominant, while coagulase negative Staphylococci (CoNS), Enterococci and Streptococci are involved less frequently **(Suchitra and Lakshmidevi, 2013).** Resistance to the chemotherapeutic antimicrobial agents is broadly classified as occurring via either intrinsic (innate) resistance or acquired resistance by horizontal gene transfer or vertical gene transfer **(Vranakis *et al.*,**

2014). Bacteria can resist the antimicrobial agents by active efflux systems and changes in cell permeability, conversion from a planktonic life cycle to a sessile biofilm life cycle**,** inactivation or enzymatic modification and/or alteration of antibiotic target **(Alekshun and Levy, 2007).** Multi-drug resistance suggests the presence of efflux pump **(Li and Nikaido, 2009).**Active efflux is now known to play a major role in the resistance of many bacterial species to antimicrobial agents**(Ahmed *et al.*, 2013)**. Bacterial efflux systems are examples of larger classes of transporters involved in the uptake of essential nutrients and ions, excretion of metabolic end products, deleterious substances and communication between cells and the environment **(Li and Nikaido, 2004).**Efflux pumps in Gram-positive bacteria belong to four unrelated families: major facilitator superfamily (MFS), small multi-drug resistance (SMR), multi-drug and toxic extrusion (MATE), and adenosine triphosphate (ATP)-binding cassette (ABC) **(Handzlik *et al,* 2013)**.

The objective of this study is to investigate the multi-drug resistance of Staphylococci isolated from surgical site infections.

MATERIALS and METHODS

Bacterial strains

One hundred Gram positive Staphylococci isolates were recovered from 190 specimens from patients with SSI admitted to Surgery Department in Zagazig University Hospitals, Egypt.

Media and chemicals

Antibiotic disks were obtained from Oxoid (Hampshire, England). These disks include ampicillin (AM, 10 μg), ampicillin-sulbactam (SAM, 20 μg), doxycycline (DO, 30 μg), tigecycline (TGC, 15 μg), gatifloxacin (GAT, 5 μg), azithromycin (AZM, 15 μg), imipenem (IPM, 10 μg), linzeolid (LZD, 30 μg), vancomycin (VA, 30 μg), clindamycin (DA, 2 μg), daptomycin (DAP, 30 μg), sulfamethoxazole-trimethoprim (SXT,25μg), amikacin (AK,30μg), levofloxacin (lev,5μg), vancomycin (VA, 30 μg), oxacillin (OX, 30 μg). The culture media Mueller Hinton (MH) agar and broth, Tryptone soya agar, Nutrient agar and broth, Mannitol salt agar and agar in dehydrated form were obtained from Oxoid (Hampshire, England).

Isolation and identification

Specimens were collected from patients with SSI admitted to Surgery Department in Zagazig University Hospitals, Zagazig, Egypt by using sterile cotton swab. After collection, swabs were seeded onto the surface of each of nutrient agar, blood agar and Mannitol salt agar plates then incubated at 37°C for 24 hours **(Winn and Koneman, 2006).**

Bacterial isolates were picked from agar plates and presumptively identified by Gram stain, colony morphology and biochemical characters according to standard microbiological techniques **(Winn and Koneman, 2006).** These tests were catalase, oxidase, coagulase, hemolysis on blood agar, mannitol fermentation and gelatin liquefaction tests**.**

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by Kirby-Bauer standard disk diffusion method. Three to five well-isolated colonies were touched with a sterile loop from an overnight agar plate culture and the growth was transferred into 5 ml of MH broth. The broth culture was incubated at 37°C with shaking for 4 to 6 hours. Turbidity was adjusted with sterile broth to obtain turbidity optically comparable to that of 0.5 McFarland standard. This results in a suspension containing approximately 1.5×10^8 CFU/ml. Within 15 minutes of preparing the adjusted inoculum, a sterile cotton swab was dipped into the inoculum, rotated several times and pressed firmly on the inside wall of the tube. The swab was streaked over the entire surface of the MH agar plate. The inoculated plates were left on a flat level surface undisturbed for 3-5 minutes. The antibiotic disks were placed

on the plates and lightly pressed into the agar. The disks were arranged at 15 mm from edge of the Petri dish and 30 mm from each other. The plates were incubated inverted at 37°C for 18 hr. The diameters of the inhibition zones were measured in mm, and interpreted as resistant, intermediate or susceptible **(CLSI, 2013).**

RESULTS

Identification of bacterial strains

One hundred Staphylococci isolates were obtained. Ninety one isolates were *Staphylococcus aureus* and nine were CoNS. The isolates were Gram positive cocci in bunches. They were confirmed biochemically as shown in **table 1.**

Table 1: Biochemical identification of *Staphylococcus aureus* & CoNS.

Biochemical Test	*Staph. aureus*	CoNS
Catalase	+	+
Oxidase	-	-
Coagulase	+	-
Hemolysis on blood agar	β-hemolysis	γ-hemolysis
Mannitol fermentation	+	-
Gelatin liquefaction	+	-
Pigmentation on nutrient agar	Golden yellow pigmentation	white colonies

Antimicrobial susceptibility profile

As shown in **table 2,** the isolates showed varied susceptibility to different antibiotics . *Staphylococcus aureus* isolates were highly resistant to tigecycline, oxacillin, ampicillin and ampicillin-sulbactam. They showed intermediate resistance to daptomycin, amikacin, azithromycin, levofloxacin, clindamycin, sulfamethoxazole-trimethoprime, doxycycline and gatifloxacin. On the other hand, they showed low resistance to vancomycin, linezolid and imipenem. CoNS isolates were highly resistant to doxycycline, oxacillin, ampicillin and ampicillin- sulbactam. They showed intermediate resistance to daptomycin, levofloxacin, clindamycin, sulfamethoxazole-trimethoprime, gatifloxacin, vancomycin, tigecycline, linezolid and imipenem, while they showed low resistance to amikacin and completely sensitive to azithromycin.

Table 2. Antibiotic resistance profile of Staphylococci isolates.

Antibiotic name	No. (%) of resistant *Staphylococcus aureus*	No. (%) of resistant CoNS
Daptomycin	26(28.6)	3(33.3)
Amikacin	21(23.1)	1(11.1)
Azithromycin	25(27.5)	0(0)
Levofloxacin	30(33)	4(44.4)
Clindamycin	53(58.2)	2(22.2)
Sulfamethoxazole-trimethoprim	43(47.3)	5(55.6)
Doxycycline	23(25.3)	6(66.7)
Gatifloxacin	30(33)	3(33.3)
Vancomycin	9(9.9)	3(33.3)
Tigecycline	91(100)	4(44.4)
Linezolid	15(16.5)	2(22.2)
Oxacillin	86(94.5)	8(88.9)
Ampicillin	87(95.6)	8(88.9)
Ampicillin-Sulbactam	71(78)	6(66.7)
Imipenem	10(11)	2(22.2)

Multi-drug resistance is defined as the resistance of microorganism to at least one member in three or more different categories of antibiotics **(Alanis, 2005)**. High rate of multi-drug resistance was found in this study. Multi-drug resistant *Staphylococcus aureus* represented 83.5% of isolates while 88.9% of CoNS were multi-drug resistant.

DISCUSSION

Surgical site infections are considered among the common healthcare-associated infection (HAIs), accounting for 31% of all HAIs among hospitalized patients **(Magill, 2012).** Despite advances in infection control practices such as improved operating room ventilation, sterilization methods, barriers, surgical technique, and antimicrobial prophylaxis, SSIs are still an important cause of morbidity, prolonged hospitalization and mortality**(Awad, 2012)**. SSI is associated with a mortality rate of 3%, and 75% of SSI-associated deaths are directly attributable to the SSI **(Awad, 2012).**

Among the Gram-positive cocci, methicillin resistant *Staphylococcus aureus* and CoNS (MRSA &MRCoNS) respectively are the most important nosocomial pathogens **(Chambers, 2001)**. Sensitivity of MRSA and MRCoNS to only a few antibacterial agents limits therapeutic options and poses a threat to the patient life **(Naqvi *et al.*, 2007).**

In the current study, *Staphylococcus aureus* and CoNS represents 48% and 0.05% of all collected specimens respectively. Also, positive cultures were found in 97.3% of specimens collected from patients. This finding was similar to that observed previously **(Agnihotri *et al.*, 2004; Mehta *et al.*, 2007)**. This may be attributed to the fact that the normal barrier function of the skin is impaired due to injury, thus allowing microbial colonization and contamination of the wounds that are almost unpreventable even with the use of topical antimicrobial agents **(Awad, 2012).**

The Staphylococci recovered from patients were identified as *Staphylococcus aureus* (91%) and CoNS (9%). Most of these bacteria are normal flora in healthy person and they can easily disseminate and cause infection when they get breaks on skins and soft tissue in any of mechanical cases **(Chambers, 2001)**. Moreover, these bacteria are commonly found in the hospital environment, which might increase wound infection rate and cross-contamination among admitted patients **(Khanal and Jha, 2010).**

In this study, *Staph. aureus* was the most predominant organism recovered from patients. This result was similar to that reported in other studies **(Ahmed *et al.*, 2014).** Staphylococcal infections are very serious and among the most frequently occurring of all antibiotic-resistant threats **(CDC, 2013).** Moreover, resistance to anti-MRSA agents usually occurs through bacterial mutation **(Rossolini *et al.*, 2014)**.

In the current study, Staphylococci isolates showed high resistance to tested β-lactams. In accordance with our findings, the studies conducted by **Ahmed *et al.*, 2013 and Perween *et al.*, 2015** reported the absolute resistance of Staphylococci isolates to β-lactams.

In this study, *Staphylococcus aureus* was highly resistant to tigecycline, while CoNS showed intermediate resistance in contrast to the finding observed by **Mewara *et al.*, 2014** in which there was no resistance for *Staph. aureus* . Moreover, 27.5% of *Staph. aureus* isolates were resistant to azithromycin, which is higher than that reported previously **(El Nakeeb *et al.*, 2014)** where 21**%** of isolates were resistant. Furthermore, *Staph. aureus* and CoNS isolates exhibited intermediate resistance to daptomycin. This was higher than that observed by **Mewara *et al.* (2014**).

In the current study, *Staphylococcus aureus* and CoNS showed low and intermediate resistance to imipenem (11% and 22.2%), respectively, which were

higher than that reported by **Abdelkarim *et al*. (2016)** who reported a resistance rate of 3.9% to imipenem.

In addition, *Staph.aureus* isolates were of low resistance to linezolid (16.5%) that is in accordance with a study performed in Menoufia University Hospitals by **Salem and Mahmoud (2014)** in which resistance rate was 1.5% only**.** On the other hand, CoNS isolates showed intermediate resistance to linezolid which is higher than that observed in previous study **(Gabr *et al*., 2016)** where no resistance to linezolid was found.

Staph. aureus and CoNS in this study showed low and intermediate resistance to vancomycin (9.9% and 33.3%, respectively). These rates were higher than that reported by **Abdelkarim *et al*. (2016)** where there was no resistance to vancomycin. Moreover, intermediate resistance to sulfamethoxazole-trimethoprime was found in all Staphylococci isolates, which is lower than that reported by **Salem and Mahmoud, 2014** in which resistance was 88.2% and higher than that found by **Abdelkarim *et al*. (2016)** where 36% of Staphylococci isolates were resistant .

Staphylococcus resistance to clindamycin observed in this study was in accordance with that reported by **Abdelkarim *et al*. (2016)** in which clindamycin resistance was 44%. This study detected intermediate resistance of *Staph. aureus* and high resistance of CoNS to doxycycline. These results were compatible with **Abdelkarim *et al*. (2016)** which reported high resistance to all Staphylococcal isolates (66%). Futhermore, this study detected intermediate resistance of *Staph. aureus* and CoNS to gatifloxacin (33% and 33.3%), respectively. This was in accordance with the results reported by **Gabr *et al*. (2016)** in which the resistance of *Staph. aureus* and CoNS were 41% and 34.5%, respectively.

Intermediate resistance was also found against both levofloxacin and amikacin. These results were compatible with those of **Gabr *et al*., 2016** In the current study, MRSA and MRCoNS isolates represented high rates of multi-drug resistance, which were 83.5% and 88.9%, respectively**.** These results were compatible with **(Song *et al*., 2001; Ahmad *et al*., 2013)**

Multi-drug resistance to antibiotics has become a serious concern for the public health setting**(Gabr *et al*., 2016)**. The role that efflux systems play in antibiotic resistance in MDR bacteria is an important subject that has been extensively discussed in recent years **(Bhardwaj, 2012)**. Although high-level resistance may not occur as a result of MDR efflux pumps alone, the association of over-expression of specific genes among highly resistant clinical isolates cannot be ignored **(Piddock, 2006).** Synergic increases in resistance seen with over-expression of efflux systems, as well as target site mutations can lead to highly resistant bacteria that are difficult to treat with the antibiotics that are currently available **(Bhardwaj, 2012).** Efflux is suspected to be the mechanism of antibiotic resistance when there is a simultaneous increase in the MICs of three or more antibiotics for a particular bacterium compared with the MICs of these antibiotics for the parent strain **(Poole, 2004).** The antibiotic resistance crisis may be attributed to the overuse and misuse of these medications, as well as a lack of new drug development by the pharmaceutical industry due to high cost and challenging regulatory requirements **(Gould and Bal, 2013).** Incorrectly prescribed antibiotics also contribute to the promotion of resistant bacteria**(Gabr *et al*., 2016)**. Studies have shown that treatment indication, choice of agent, or duration of antibiotic therapy is incorrect in 30% to 50% of cases **(CDC, 2013).**

In conclusion, this study suggests the application of a strict antibiotic dispensing policy that is based on sensitivity testing and decreasing the use of broad spectrum

antibiotics in order to decrease the emergence of multi-drug resistant Staphylococci.

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انتشار الميكروبات العنقوديه ذات المقاومه المتعدده للأدويه المعزوله من عدوى المواضع الجراحيه

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تعتبر عدوى المواضع الجراحيه واحدة من أكثر المشاكل الشائعة والمدمرة. فهي تمثل مشاكل صحية عامة خطيرة عالميا متمثلة في ارتفاع معدل الوفيات سنويا. تمثل عدوى المواضع الجراحيه المضاعفات العظمي بعد معظم العمليات الجراحيه. لقد استهدفت هذه الدراسة التحقيق في المقاومة المتعددة للمضادات الحيويه بين الميكروبات العنقوديه المعزولة من عدوى المواضع الجراحيه.

تم إجراء هذه الدراسة علي ١٠٠ عينة من الميكروبات العنقوديه تم تجميعها من قسم الجراحه بمستشفيات جامعة الزقازيق مصر. وقد تم جمع جميع العينات في ظروف معقمة وتم نقلها إلى معمل الميكروبيولوجي بكلية الصيدلة جامعة الزقازيق، حيث تم التعامل معها على الفور.هذا وكانت الميكروبات المعزولة هى استفيلوكوكس أوريس (٩١%)و استفيلوكوكس السالبة لانزيم التخثر(٩%).

تم اختبار حساسية كل الميكروبات المعزولة للمضادات الحيوية المختلفة بطريقة انتشار القرص(الديسك) المعياري (كيربي باور). وكان الفانكوميسن ، اللينزوليد، الاميبينم،الأميكاسين،الأزيثرومايسين والدابتوميسن هم أكثر المضادات الميكروبية

فاعلية ضد العزلات. وعلي النقيض من ذلك كانت البيتالاكتام واتحادات البيتالاكتام مع مثبطات بيتالاكتامازيزوكذلك التايجسيكلين هم المضادات الحيوية الأقل فاعلية ضد كل الميكروبات المختبرة.
تم الكشف عن المقاومة المتعددة للأدوية على أنه فقدان الحساسية لعامل واحد على الأقل في ثلاثة أو أكثر من فئات مضادات الميكروبات. وقد لوحظت هذه المقاومة المتعددة للمضادات الحيوية في
٨٣,٥ % و ٨٨,٩% من عزلات استفيلوكوكس أوريس وعزلات استفيلوكوكس السالبة لانزيم التخثرعلى التوالى. وتعتبر هذه النسبه العاليه مؤشر خطير وجرس انزار بسبب الإستخدام السيء
للمضادات الحيويه. ومن النتائج التي تم الحصول عليها تقترح هذه الدراسة تطبيق سياسة صارمة لتوزيع المضادات الحيوية تعتمد على اختبار الحساسية وتقليل استخدام المضادات الحيوية واسعة الطيف من أجل تقليل
ظهور مقاومة متعددة للأدوية