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## Detection of Bacterial Pathogen from Wound Infections and Study Some of their Virulence Factors

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### Abstract

Wound infections are one of the most important and potentially serious complications that occur in the acute period following injury. In this study, (35) wound swap were collected from (35) patients with suspected wound infection, during the period from December (2017) to March (2018). The results of this study revealed that (74.29%) of samples was positive for bacterial culture, while (25.71%) of sample have no growth. The bacterial culture shown that (38.46%) (10 isolate) of bacterial isolates was *Staphylococcus aureus* followed by *Pseudomonas spp.* (19.23%) (5 isolate), *E. coli* (19.23%) (5 isolate), *Klebsiella* (15.38%) (4 isolate) and *Proteus* (7.69%) (2 isolate). In-vitro, the susceptibility of bacteria towards some antibiotics included in this study revealed that the Ciprofloxacin, are the most effective antibiotics to different types of pathogenic bacteria, while all bacteria was resistance to Ampicillin.

**Key Words:** Wound swap, bacterial infection, virulence factor, antibiotics

### Introduction

Wound infection is a common problem (Giacometti *et al.*, 2000). Infection of the wound happened due to entry of the bacteria through breached skin. These bacteria stop healing and produce sign and symptoms. Multiple organisms can cause wound infection (Melling *et al.*, 2001). Most wound infections can be classified into two major categories: skin and soft tissue infections, although they often overlap as a consequence of disease progression. Infections of hospital-acquired wounds are among the leading nosocomial causes of morbidity and increasing medical expense (Cutting and White, 2004).

Diagnosis of wound infection is an important part of wound care, as infection can cause a wound to stall in the healing process, or even enlarge. The signs of infection (redness, swelling, increased drainage, increased pain) and be proactive in assessing and diagnosing infection.

Swab cultures are the most commonly used method to culture wound infections due to the fact that they are easy to perform, do not cause trauma to the wound bed and avoid a surgical procedure that can result in complications. Swab cultures, sample the wound surface, along with small amounts of wound fluid expressed from the wound or obtained from areas of tunneling or undermining, in order to quantify the

amount/number and type of bacteria present on a wound (the wound needs to be cleansed before swabbing takes place) (Meyers, 2008).

For the treatment of infection a large number of antibiotics are used. Both broad spectrum and narrow spectrum antibiotics are available nowadays. It is ideal to give proper antibiotic after culture and sensitivity of the wound swab, pus or infected tissue. Unfortunately this practice is uncommon among the physicians especially of the developing countries. Improper and irrational use of antibiotics and genetic and non-genetic drug resistant mechanisms of bacteria lead to drug resistance. Drug resistant bacteria are the most important therapeutic challenge in the field of infectious diseases, many of them are multi drug resistant (Atiyeh *et al.*, 2007).

**2. Material and Methods**

**Table (3–1)** Type of virulence factors detected in bacterial isolates

Bacterial isolates	capsule	hemolysin	Protease	CFA I	CFA III
<i>S.aureus</i>	+	+	+	-	+
<i>Pseudomonas</i>	+	+	+	-	-
<i>Klebsiella spp.</i>	+	-	+(3)	+	+
<i>E.coli</i>	+	+	+	+	+
<i>Proteus</i>	-	+	+	-	+

The ability of *S.aureus* isolate to produce some virulence factors was investigated and the results are shown in table (3-1). *S. aureus* isolates had shown to possess the polysaccharide capsule which was an important component in the pathogenesis, and enhances bacterial virulence by modulate *S. aureus* adherence to endothelial surface in vitro, this agree with result mentioned by Nair *et al.*, (2000).

In this study, *S. aureus* isolate (100%) had the ability to produce the hemolysin, and the type of hemolysis was Beta-hemolysis due to the

**2.1. Patient**

This study includes 35 patients with wound infection during the period from December (2017) to March (2018). The samples were collected using sterile cotton swabs from patients clinically diagnosed with wound infection.

**2.2. Identification of isolated bacteria**

Identification of isolated bacteria was carried out by using culture methods and biochemical tests. The samples was first culture on Blood agar and MacConkey agar then incubated for (24 h. at 37 °c) after that the bacterial growth was diagnosed by using specific culture and biochemical tests.

**3. Result and Discussion**

**3.1. Detection of virulence factors**

The ability of different types of bacterial isolates to produce some virulence factors was investigated and the results are shown in Table (3-1)

presence of a clear zone of hemolysis around the bacterial colonies formed on human blood agar, this agree with result mentioned by Dinges *et al.*, (2000).

*S. aureus* produce protease as an important virulence factor. This result show (100%) of *S. aureus* isolates produced extra cellular protease as shown in table (3-1), this agree with Karlsson and Arvidson, (2002) who pointed to production of four major extracellular protease by *S. aureus* (serine protease , cysteine protease , metalloprotease and

staphopain) which are important virulence factors and controlling the gene expression for this enzyme by regulator factors.

The ability of *Staphylococcus aureus* to produce colonization factor antigen was tested and it was noted that (100%) of isolate could produce colonization factor antigen III (CFA III).

*E. coli* isolate were tested for their ability to produce capsule and it was found that (100%) from these isolate could produce the polysaccharide capsule. Silver and Vimr, (1990) demonstrated that some capsules have been correlated with pathogenic strains of *E. coli*.

Hemolysin production by *E. coli* was studied, and it was found that (100%) of isolate was able to produce  $\alpha$ -hemolysin on blood agar plate.

These results agree with Herlax *et al.*, (2010) who pointed that  $\alpha$  – hemolysin (Hly A) is an extracellular toxin secreted by *E. coli*, targeting to plasma membranes of eukaryotic cells. It has been found that this toxin is released to external media associated to bacterial outer membrane vesicles (OMVs).

The erythrocytes are not only affected by hemolysin, but also leucocytes (white blood cells). *E. coli* hemolysin is potentially cytotoxic to monocytes, lymphocytes and macrophages leading them to autolysis and death (Al-Hasson, 2010).

In regard to protease, the ability of *E. coli* to produce extracellular protease in M<sub>9</sub> media was investigated, and it was found that all these isolates (100%) had the ability to produce extracellular protease after 24 hours of incubation table (3-1). A clear halo of transparent area was found around the

colony after the addition of 3 ml of 3% trichloroacetic acid. This result was identical with that obtained by Baron, (1994). Extracellular protease plays an important role in the cell survival and cell-cell communication (Abhrajyoti *et al.*, 2009).

The ability of *E. coli* to produce colonization factor antigen was tested and it was noted that (100%) of isolate could produce colonization factor antigen I (CFA I) and colonization factor antigen III.

The ability of *Klebsiella spp.* isolates to produce some virulence factors was investigated and the results are shown in table (3-1). From these results, it could be noticed that all *Klebsiella spp.* isolates possessed the polysaccharide capsule which surround the bacterial cell. This result agrees with the result obtained by Podschun and Ulmann, (1998) who had pointed that the diagnostic feature for *Klebsiella* is capsular polysaccharide, and it's essential virulence factor for *K. pneumoniae*.

The results also showed that all *Klebsiella spp.* isolates could not produce hemolysin extracellularly when cultured on blood agar, and the type of hemolysis was gamma hemolysis because there was no hemolysis present on blood agar plates.

*Klebsiella spp.* isolates were positive (75%) (3 isolates) to produce extracellular protease. These results agreed with Bondarenko *et al.*, (2000) who found that (58.6%) of *Klebsiella* are with the presence of SIg-A protease activity, which made this enzyme to play an important role in the pathogenicity of this bacteria.

The ability of these bacteria to produce CFAI, CFAlII was studied, CFA/1 was investigated

in the presence of D-mannose and human erythrocyte group A, and it was found that all isolates (100%) have ability to produce CFAI and CFaIII. This means that, it is so called mannose resistant hemagglutination-assay positive, however, the presence of mannose resistant adhesion factor may help in the bacteria in adhesion to the epithelial cells, and then enhance the pathogenicity of bacteria(AL-zaag.,1994).

Also *Pseudomonas* spp. was tested for capsule and protease production and the result show that all isolate was positive for capsule production and protease. Surface structures, including pili and the polysaccharide capsule or glycocalyx, appear to

mediate the initial attachment of *P. aeruginosa* to its prospective host, thus permitting colonization. Extracellular enzymes such as alkaline protease, elastase, phospholipase C, and exotoxin A degrade infected tissues and promote bacterial invasion (Pollack, 1984).

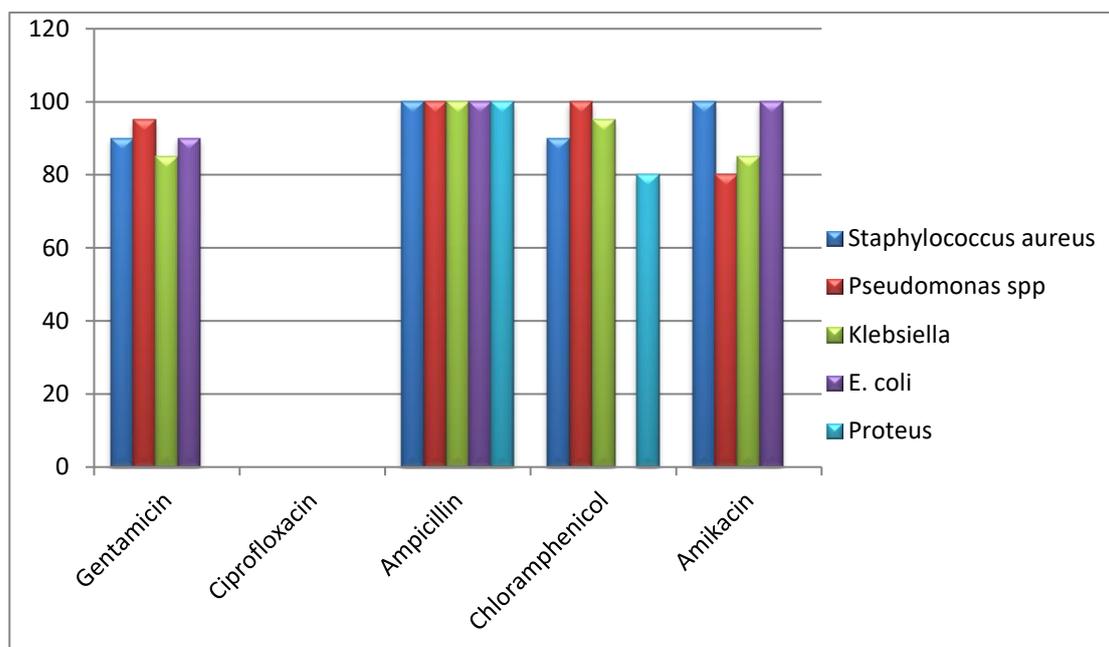
### 3.2. Antimicrobial susceptibility pattern of bacterial isolates

The susceptibility of the recovered bacterial isolates to antibiotics was determined using disc diffusion assay, Kirby-Bauer method. The results obtained showed that the organisms varied in their susceptibility to all the antimicrobials used. Majority of them showed multi-resistances.

**Table (3-2)** Antimicrobial susceptibility patterns of bacterial isolates

Bacterial isolates	Antibiotics				
	CN	CIP	C	AM	AK
<i>Staphylococcus aureus</i>	R	S	R	R	R
<i>Pseudomonas</i> spp	R	S	R	R	R
<i>Klebsiella</i>	R	S	R	R	R
<i>E. coli</i>	R	S	S	R	R
<i>Proteus</i>	S	S	R	R	S

CN: Gentamicin, CIP: Ciprofloxacin, , AM: Ampicillin, C: Chloramphenicol, AK: Amikacin



Among the isolated pathogens all of them shows 100% sensitivity towards Ciprofloxacin. While bacteria show different resistance rate to Amikacin, Gentamycin and Chloramphenicol. While all isolates were resistance 100% toward Ampicillin.

All of the isolated bacteria were resistant to ampicillin. This may be due to the antibiotics having been in use for much longer time and their oral route of administration that affects their rate of absorption into blood stream.

The results of antimicrobial sensitivity showed that *S. aureus* was highly resistant for most of the antibiotics tested, while it had less resistance to ciprofloxacin. The adaptation of *S. aureus* to the modern hospital environment has been marked by the acquisition of drug resistance genes soon after antibiotic introduction (Bouchillon *et al.*, 2004).

Also the results showed that *Pseudomonas* spp was highly resistant for most of the antibiotics tested. This result was in agreement with study done by Manyahi, (2012).

Increasing resistance to various anti-*Pseudomonas* agents has been reported worldwide and this poses a serious problem in therapeutic management of *P. aeruginosa* infections (Brown and Izundu, 2004).

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