Effect of most common antibiotics against bacteria isolated from surgical wounds in Aden governorate hospitals, Yemen

Abstract:

Surgical wound infections by pathogenic bacteria and increasing antibiotics resistance are of the most serious health threats facing the patients especially in developing countries like Yemen. The main objective of this work was to identify the common pathogenic bacteria infecting surgical wounds and their resistance to common used antibiotics. One hundred and twenty wound swabs were collected from surgical wound patients at Aden City, Yemen. The pathogenic bacteria were isolated and identified according to standard microbiological methods. Also, antibiotics susceptibility tests were performed using disk diffusion technique. The results showed that out of 120 samples, 68 (56.67%) showed bacterial growth. Overall, *Staphylococcus aureus* was the predominant isolate 27 (39.70%) followed by *Escherichia coli* 19 (27.94%), *Pseudomonas aeruginosa* 13 (19.12%), and *Proteus mirabilis* 9 (13.24%). All isolated bacteria were found to be highly resistant to commonly used antibiotics. *Staph. aureus* was most susceptible to cefotaxime, vancomycin, and ciprofloxacin and highly resistance to ceftazidime, nalidixic acid, erythromycin, and tetracycline. The *E. coli* isolates showed resistance (100%) to vancomycin and tetracycline and moderately sensitive to ceftazidime and gentamycin. *P. aeruginosa* showed from high to moderate resistance to most tested antibiotics except gentamycin and cefotaxime. Most of all the *P. mirabilis* isolates were sensitive to ceftazidime, cefotaxime, ciprofloxacin and gentamycin and highly resistance to amoxicillin, erythromycin, and vancomycin. The current study findings that the reduced sensitivity of isolated bacteria to commonly used antibiotics is an alarming increase of infections caused by antibiotic-resistant bacteria.

Key words: Antibiotics, Pathogenic bacteria, Resistant, Wounds infection

Introduction

Wound infections are one of the most common nosocomial infections and are an important cause of morbidity and account for 70-80% mortality[1,2]. Wound infections can be caused by a variety of microorganisms ranging from bacteria to fungi and parasites as well as virus[3]. The most common bacterial genera infecting wounds are *Enterococci*, *Escherichia*, *Pseudomonas, Klebsiella, Enterobacter, Proteus* and *Acinetobacter* [4,5].

Advances in the control of wound infections has become more challenging due to widespread bacterial resistance to antibiotics, and to a greater incidence of infections caused by methicillin-resistance *Staph. aureus* and polymicrobic flora[6].

Antimicrobial resistance problem is challenging in low income countries due to high prevalence of infections, irrational uses of antimicrobials, over the counter availability of drugs and lack of clinical microbiology laboratories for antimicrobial susceptibility testing[7]. Antimicrobial resistance can increase complications and costs associated with procedures and treatment[8].

However, very limited data are available on the kinds of bacterial isolates and their drug resistance profile associated with wound infection in Aden Hospitals, Yemen. Therefore, the present study was carried out to isolate and identify the pathogenic bacteria from surgical wound infections and determination of their susceptibility to various common antibiotics.

Materials and Methods

Samples Collection

Wounds samples were collected from one hundred and twenty (120) patients that undergo surgical operation in three general hospitals, Algomhori, Khalifa, and Alsadaka, in Aden City of Yemen. The wound samples were collected by using a sterile cotton swab, the superficial, medium or deep of the infected area was swabbed gently and then the swabs were transported to the laboratory. Each sample was inoculated on McConkey agar, Nutrient agar and Blood agar (Himedia, India) and then incubated aerobically at 37°C for 24 h.

Identification of isolated bacteria

All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures according to the criteria of Bergey's Manual of Systematic Bacteriology, 2nd edition[9].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done on Mueller–Hinton agar (Oxoid, England) using disk diffusion technique according to Kirby–Bauer method[10]. Ten types of antimicrobial agents tested were: Amoxycillin (30 µg), Ceftazidime (30 µg), Vancomycin (30 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), Nitrofurantion (30 µg), tetracycline (30 µg), erythromycin (15 µg), Nalidioxic acid, (30 µg), gentamicin (10 µg) (Himedia, India). The plates were incubated at 37°C for 18-24 hrs. Zones of inhibition were measured in millimeters using a caliper.

Results

The result from the current study revealed that the 120 samples were collected from both sexes (males and female) with surgical wounds infection. 88 samples (73.33%) from males and 32 samples (26.67%) from females as shown in Table (1).

Table 1: The number of samples distribution between genders

No.	Genders	Number of samples	Percentage %
1	**Males**	88	73.33%
2	**Females**	32	26.67%
Total		**120**	**100%**

From the results, there was only 68 samples (56.67%) showed as a positive growth in culture media and 52 samples (43.33%) were reported as negative growth in culture media (Table 2). **Table 2:** The sample positive and negative growth in media

Type of growth	Number of sample	Percentage %
Positive growth	68	**56.67%**
Negative growth	52	43.33%
Total	**120**	**100%**

The table (3) showed that *Staph. aureus* at 27 (39.70%) was the predominant isolate followed by *E. coli* at 19 (27.94%), *P. aeruginosa* at 13 (19.12%), and *P. mirabilis* at 9 (13.24%).

Table 3: The various species of bacteria isolated from wound culture and their frequency

Bacterial species	Frequency of isolation	Percentage %
Staph. aureus	27	39.70%
E. coli	19	27.94%
P. aeruginosa	13	19.12%
P. mirabilis	9	13.24%
Total	**68**	**100 %**

The antibiotic sensitivity of isolated bacterial strains was carried out by Kirby-Bauer disk diffusion assay against ten antibiotics. In this study, the *Staph. aureus* isolates showed high resistance against ceftazidime (100%) followed by nalidixic acid (88.9%), tetracycline (85.19%), and erythromycin (74.1%). The medium-resistant of *Staph. aureus* was recorded to amoxicillin at 51.9% and nitrofurantoin at 48.1%. *Staph. aureus* showed very high sensitivity to cefotaxime (100%) followed by vancomycin (96.3%) and ciprofloxacin (85.1%) as shown in table (4).

Table 4: Resistance and sensitivity pattern of isolated *Staph. aureus*

Antibiotics	Resistant	Sensitive	Total
Amoxicillin	14(51.9%)	13 (48.1%)	27
Cefotaxime	0	27(100%)	27
Ceftazidime	27(100%)	0	27
Ciprofloxacin	4(14.9%)	23(85.1%)	27
Erythromycin	20(74.1%)	7(25.9%)	27
Gentamycin	10(37.0%)	17(63.0%)	27
Nalidixic acid	24(88.9%)	3(11.1%)	27
Nitrofurantoin	12(48.1%)	15(51.9)	27
Tetracyclin	23(85.19%)	4(14.81%)	27
Vancomycin	1(3.7%)	26(96.3%)	27

The isolated *E. coli* from wounds indicated 100% resistant to vancomycin and tetracycline. Also, *E. coli* showed high resistance nalidixic acid (73.7%), erythromycin and amoxicillin (68.4%), nitrofurantoin and cefotaxime (63.2%). It was moderately resistant to gentamycin at 36.8% as listed in table (5).

Table 5: Resistance and sensitivity pattern of isolated *E. coli*

Antibiotics	Resistant	Sensitive	Total
Amoxicillin	13(68.4%)	6(31.6%)	19
Cefotaxime	12(63.2%)	7(36.8%)	19
Ceftazidime	10(52.6%)	9(47.4%)	19
Ciprofloxacin	7(36.9%)	12(63.1%)	19
Erythromycin	13(68.4%)	6(31.6%)	19
Gentamycin	7(36.8%)	12(63.2%)	19
Nalidixic acid	14(73.7%)	5(26.3%)	19
Nitrofurantoin	12(63.2%)	7(36.8%)	19
Tetracycline	19(100%)	0	19
Vancomycin	19(100%)	0	19

P. aeruginosa showed highly resistance to amoxicillin and vancomycin at 100%, followed by tetracycline at 92.3%, erythromycin at 84.6%, nalidixic acid and nitrofurantoin at 76.9%, ciprofloxacin at 69.2%. *P. aeruginosa* was sensitive to gentamycin (76.9) and cefotaxime (69.2) as shown in table (6).

Table 6: Resistance and sensitivity pattern of isolated *P. aeruginosa*

Antibiotics	Resistant	Sensitive	Total
Amoxicillin	13(100%)	0	13
Cefotaxime	4(30.8%)	9(69.2%)	13
Ceftazidime	6(46.2%)	7(53.8%)	13
Ciprofloxacin	9(69.2%)	4(30.8%)	13
Erythromycin	11(84.6%)	2(15.4%)	13
Gentamycin	3(23.1%)	10(76.9%)	13
Nalidixic acid	10(76.9%)	3(23.1%)	13
Nitrofurantoin	10(76.9%)	3(23.1%)	13
Tetracycline	12(92.3%)	1(7.7%)	13
Vancomycin	13(100%)	0	13

The *P. mirabilis* isolates showed sensitive to ceftazidime at 88.9% followed by cefotaxime ciprofloxacin and gentamycin at 77.8% for each. Most of the *P. mirabilis* were highly resistance to amoxicillin, erythromycin, and vancomycin at 100% and followed by nitrofurantoin at 88.9% as listed in table (7).

Table 7: Resistance and sensitivity pattern of isolated *P. mirabilis*

Antibiotics	Resistant	Sensitive	Total
Amoxicillin	9(100%)	0	9
Cefotaxime	2(22.2%)	7(77.8%)	9
Ceftazidime	1(11.1%)	8(88.9%)	9
Ciprofloxacin	2(22.2%)	7(77.8%)	9
Erythromycin	9(100%)	0	9
Gentamycin	2(22.2%)	7(77.8%)	9
Nalidixic acid	6(66.7%)	3(33.3%)	9
Nitrofurantoin	8(88.9%)	1(11.1%)	9
Tetracycline	4(44.4%)	5(55.6)	9
Vancomycin	9(100%)	0	9

Discussion

Bacterial wound contamination is a serious problem in the hospital and the treatment of wound infections remain a significant concern for surgeons. The risk of developing wound infection depends on the number of bacteria colonies on the wound. The problem has been magnified due to the unrestrained and rapidly spreading resistance to the available array of antimicrobial agents[11]. The present study, it was revealed that the 88 samples (73.33%) were collected from males and 32 samples (26.67%) from females. This result is similar with findings by Anthony *et al.*[12] who recorded that the samples were collected from patients with wound infection was up of 40 males and 24 females.

Of 120 samples processed, 68 samples (56.67%) showed as a positive growth in culture media and 52 samples (43.33%) were reported as negative growth. In study by, Farrag *et al.*[11] revealed that the 41 samples (82%) collected from wound infection were recorded as a positive bacterial growth and only 9 samples were recorded as negative bacterial growth.

Wounds are known to be easy portals for infection and provides suitable medium for the proliferation of microbial organisms, so both of gram positive and gram-negative bacteria are known to cause wound sepsis. In the present, four of pathogenic bacteria that isolated from 68 morbidity condition cause infections wounds surgeries were identified. These pathogenic bacteria are *Staph. aureus*, *E. coli*, *P. aeruginosa*, and *P. mirabilis*

The results showed that *Staph. aureus* was the predominant (39.70%) followed by *E. coli* (27.94%), *P. aeruginosa* (19.12%), and *P. mirabilis* (13.24%). In similar study by, Tayfour *et al.*[4] showed that the *Staph. aureus* bacteria was the most bacteria isolated from King Fahd Hospital patients with 33.5% percentage. A study by Anthony *et al.*[12] revealed that the *Staph. aureus* was the predominant bacteria (25%), followed by *P. aeruginosa* (20%), *E. coli* (15%), and *P. mirabilis* (10%).

Staph. aureus exists naturally on the skin surface by 40-60% of healthy people as well as present in the hospital environment. It is the main cause of infection in public hospitals, and the role of convalescence, and hospitals that provide health care for acute cases[13]. Poor wound management allows the bacteria to invade the inner tissue and bring about chronic systemic infection[14].

The *P. aeruginosa* bacteria are common in hospitals and the presence of diseases associated with hospital-acquired infection that are transmitted saluting this type of bacteria, mainly from non-living sources to the body's tissues by disinfectants and surgical instruments used[15].

The *E. coli* bacteria that normally lives in the humans colon and is often cause infections of wounds contaminated with urine and feces, as well as causing appendicitis and bile duct inflammation. These bacteria invasion of the wound is a clear case of poor hospital hygiene, just like other implicated bacteria are frequent agent of nosocomial infection[16].

The *P. mirabilis* bacteria was found in hospitals and it has an active role in bringing about infections of wounds and burns[15]. Most of the bacteria that exhibited higher rates of antibacterial resistance are human normal flora and biofilm forming pathogens such as *Staph. aureus, P. aeruginosa,* and *E. coli*[17].

In this study, the *Staph. aureus* showed high resistance against to many antibiotics that used to treat the *Staph. aureus* infection such as erythromycin and tetracycline. In contrast, *Staph. aureus* showed very high sensitivity to cefotaxime, vancomycin, and ciprofloxacin. This finding is in agreement with the work of Adcock *et al.*[18] and Sani *et al.*[19] who reported that clinical *Staphylococci* are resistant to multiple antibiotics.

Staph. aureus rapidly develop resistance to many antimicrobial drugs particularly that used singly for treatment of chronic infection. Resistance to drugs of the erythromycin group tends to emerge so rapidly that these drugs should not be used singly for treatment of chronic infection. Drug resistance tetracyclines and erythromycins determined by plasmids can be transmitted among staphylococci by transduction and perhaps by conjugation[20].

The *E. coli* isolated from wounds indicated 100% resistant to vancomycin and tetracycline. Also, *E. coli* exhibited high resistance to nalidixic acid (73.7%), erythromycin and amoxicillin (68.4%), nitrofurantoin and cefotaxime (63.2%). This finding is in agreement with the work of Adwan *et al.*[21] who documented that the *E. coli* showed resistance to different antibiotics which used to treat it infection. Also, in this study, *E. coli* was moderately resistant to gentamycin inhibitory (36.8%). This is consistent with results of Giacometti *et al.*[13] who reported that *E. coli* was resistant to gentamycin at 50% and ciprofloxacin at 36.7%.

In present study *P. aeruginosa* showed reduce sensitivity to commonly used antibiotics except ciprofloxacin (69%), ceftazidime (83.3%) and ciprofloxacin (69.2%) has been stated to be the most potent oral drug available for the treatment of *P. aeruginosa* infections. Similar reduced resistance of *P. aeruginosa* to ciprofloxacin has been reported in India[22]. Also, a similar result was documented from other study[23]. On the other hand, *P. aeruginosa* showed high level of resistance (87.5%) to gentamicin and considerable level of resistance (57%) to tetracycline[24]. It is undoubtable that at the present time, the oral drug ciprofloxacin, are the most effective antibiotics against *P. aeruginosa* involved in wound infection relative to most other commonly used drugs.

P. aeruginosa causes infection in all parts of the human body. The bacterium is naturally resistant to a wide range of antibiotics which is attributable to its resistance mechanisms such as efflux pumps and the ability to form biofilm that reduces further *P. aeruginosa* susceptibility to antibiotics[17]. The presence of such biofilm greatly contributes to persistent bacterial infections in surgical sites because of their inherent high tolerance to all antimicrobials and immune cells[25].

The *P. mirabilis* isolates revealed sensitive to ceftazidime at 88.9% and cefotaxime ciprofloxacin and gentamycin at 77.8% for each. Most of the *P. mirabilis* were highly resistance to amoxicillin, erythromycin, and vancomycin at 100% and followed by nitrofurantoin at 88.9%. This results agree with the studies carried out by Mordi and Momoh[26] and Manikandan and Amsath[27].

Conclusions

In conclusion, the increase of isolated bacteria resistance to used antibiotics resulting from uncontrolled, extensive incorrect and misuse of these agents in hospitals as well as in the country as a whole. This is promoted by the lack of national antibiotic policy and over-the-counter antibiotic availability in Yemen. Hence, it is essential to introduce an effective national and state level antibiotic policy and draft guidelines to preserve the effectiveness of antibiotics and for better patient management.

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