



Susceptibility and Resistance Patterns of Bacteria Isolated from Infected Wounds, Burn, Medical Device Tips and Blood to Doxycycline and Septrin from Patients in the City of Benghazi

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Authors' contributions

This work was carried out in collaboration among all authors. All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, took part in drafting the article or revising it critically for important intellectual content, gave final approval of the version to be published and agree to be accountable for all aspects of the work.

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ABSTRACT

Wound infections and septicemia with drug-resistant bacteria lead to higher mortality and morbidity and increased healthcare costs. We aimed to evaluate the prevalence rate of nosocomial bacterial agents with antimicrobial susceptibility patterns in hospitalized and non-hospitalized patients. Samples are collected from the wound pus, burn; medical device tip and blood of male and female patients and are sent for culture and sensitivity to the Microbiology Department of Al Saleem medical laboratory, Benghazi, Libya, from October 2021 to January 2022. Collected blood samples were directly inoculated into brain heart infusion (BHI) broth, while various swabs were collected by Transport medium and both are cultured on Blood agar, Chocolate agar, MacConkey - aerobic and anaerobic blood agar at 35°C, with CO₂. The wound pus was the most samples from which bacteria were isolated, followed by medical device tip swabs, and the least isolation was from a blood specimen. 47 of the specimens were culture-positive. Of these *Staph aureus*, Strep pneumonia and *E. coli* were the most abundant bacterial identified in clinical samples. According to susceptibility testing results, antibiotic resistance patterns of Gram-negative bacteria showed that the highest resistance rate was against Septrin (74.4%) and the lowest rate, 17.8%, was related to Doxycycline (44.6%) respectively. The isolates from the nosocomial infection in these patients are resistant to Septrin and Doxycycline antibiotics. We recommend that whenever the diagnosis of nosocomial infection is made, a more effective antibiotic treatment be instituted until the susceptibility of the strain is identified.

Keywords: Doxycycline; nosocomial infection; pus swab; *Staph aureus*.

1. INTRODUCTION

Antibiotic resistance, and the extent to which bacteria are able to resist drugs manufactured to kill them, is one of the biggest challenges to public health today. Antibiotics are among the most importance tools that used in the fight against life-threatening infections. Nosocomial infections have called (hospital-acquired infections) and refer to infections that occur within 48 hours of a patient's admission to hospital, three days of a patient's discharge from hospital, or within 30 days of an operation [1,2].

Moreover, this infection includes occupational injuries among medical staff in medical facilities [3], as well as invasive devices such as ventilators used in modern health care and catheters are associated with this infection [4]. A wound can be defined as any damage or injury to the skin that limits its protective function. This acute damage generally caused by external injuries to the skin, such as sharp cuts, minor cuts, insect and animal bites, burns caused by heat, friction, cold, chemicals, lacerations, puncture wounds, and surgical incisions [5] as well as the presence of necrotic tissue in the skin. Wound - this can aid the growth of bacteria, especially anaerobic [1,6] All exposed skin wounds are colonized by bacteria, but this does not mean that all wounds are infected.

As infections may occur in wounds during healing, regardless of whether they are infected

or not, a certain level of swelling and increased warmth at the wound site is normal and should not be confused with a clinical infection. When the skin cracks, its protective defense mechanisms become weak, and the environment prepared to become more conducive to infection with bacteria and thus their number increases.

These bacteria come from three essential sources; the environment (such as dirt, dust, foreign bodies, and bacteria on clothing, hands, and medical equipment). The surrounding skin (where normal skin contains bacteria, referred to as symbionts) and the mucous membranes (the digestive system, mouth, and urogenital system) [5]. Septicemia occurs when the infection spreads to all areas of the body via the bloodstreams and causes symptoms such as chills, fever, and tachycardia [5,7].

Several studies have indicated that adult bloodstream infection belongs to microorganisms of the family Enterobacteriales (*Klebsiella* spp and *Escherichia coli*) and *Enterococcus* spp [8].

Other studies have also indicated that most bacterial infections are caused by Gram-positive bacteria coagulase-negative staphylococci (CoNS), *Staphylococcus aureus* and *Enterococcus faecalis* (13%) [9]. Thus, the study aims to determine the association of factors Bacteremia in hospitals with a pattern of antimicrobial susceptibility in inpatients and non-resident patients.

2. MATERIALS AND METHODS

2.1 Study Area

This cross-sectional study was conducted at Microbiology Department of Al-Saleem laboratory, Benghazi, Libya, from October 2021 to January 2022. The samples assembled from the wounds pus, burn; medical devices, tips and blood from male and female patients in the age group of 3 to 86 years with clinical characteristics and septicemia suspected to be infected.

2.2 Sampling

All swabs specimens were collected aseptically and carried from patients by Stuart's, Amies. Collected blood samples were directly inoculated into brain heart infusion (BHI) broth with the blood to broth ratio of 1:10 for up to 5 days and were immediately transported to the microbiology department. Age, sex, health status and use of medicines, infection type were recorded for all included patients.

2.3 Blood Cultures

Positive culture samples were directly inoculated onto blood agar, chocolate agar, and MacConkey agar and incubated overnight at 37°C aerobically. Blood agar and Chocolate plates were incubated at 35°C in microaerophilic conditions (containing 5% CO₂). Moreover, 0.5 to 1 mL of the swab specimen was inoculated into a tube of enriched thioglycolate broth [9,10].

2.4 Determination of Bacterial Isolates

Visual investigation of the bacterial colonies shows culture positivity. The supposed organism is then defined by Gram's stain and a series of biochemical tests, including an oxidase test, catalase test, Carbohydrate utilization test, Citrate utilization test, DNAase test, urease test, coagulase test, Analytical profile index (api) 20e test and Mannitol Salt Agar [9,11-14].

2.4.1 Direct Vitek 2 AST

The supposed strains' morphologically similar colonies were determined by biochemical assays utilising the Vitek system (bioMerieux, Hazelwood, MO). In brief, the cell density of the remaining bacterial suspension was modified to a density of 0.5 McFarland after dilution in 0.45% saline; 145µL of the bacterial suspension was drawn into 3 mL of 0.45% saline solution to further adjust the bacterial cell density. Vitek

cards were inoculated with suspension vials and loaded into the Vitek 2 automated reader-incubator. Vitek cards AST-GN13, AST-Gp67, and AST-Gp68 were utilized for gram-negative bacteria, *staphylococci/enterococci/ streptococci* and *Strep pneumoniae*, respectively [15].

2.5 Antimicrobial Susceptibility Testing

Susceptibility of bacterial isolates to antibacterial drugs was carried out by the disk diffusion method to the following antibiotics: Doxycycline-DO (30 µg) and Seprine- SXT (25 µg). Analysis of the results was carried out following the recommendations of CLSI [16]; Kassam et al. [17]. In brief, 4–6 morphologically similar bacteria colonies from clinical samples were collected with an inoculating loop and relocated into a tube including 5 mL of broth culture, gradually and carefully mixed until a relatively homogenous suspension was obtained, and incubated at 37°C. A sterile non-toxic dry cotton swab was utilized to streak a sample of the centralized inoculation on the Mueller–Hinton agar plate. With the lid on, the inoculums were allowed to dry for 5–15 minutes.

The antibiotic disks were distributed to the plates with sterile forceps at a distance of 15 mm from the edge and 24 mm apart from each other. After 24 hours of incubation at 37°C, the diameters of the zones of bacterial growth inhibition around the disks were measured to the nearest millimetre. The isolates were classified as sensitive (S), or resistant (R) utilising a standardized table. The inhibition zone of a ruler was measured in millimetres [16].

The antibiotics tested were Seprin (300 µg; Resistant ≤12; Sensitive ≥17) and Doxycycline-DO (30 µg; Resistant ≤12; Sensitive ≥16).

2.6 Data Analysis

The data was analyzed by SPSS programs version 20.

3. RESULTS

3.1 Distribution Table of Different Wound Pus and Blood Infection by Genders

The gender distributions of the patients with swab infection are reported in Table 1. Concerning gender, the swab infection rate was higher in females than in males.

Table 1. Distribution table of different wound pus and blood Infections by genders

Sex	Frequency	Percent
Female	25	53.2
Male	22	46.8
Total	47	100.0

3.2 Distribution of Positive Cases by Age Groups

Regarding age distribution, most of the positive patients were identified in the 45-65 age groups, followed by the 24-44 age groups.

Table 2. Distribution of positive cases by age groups

Age	Frequency	Percent
3-23 years	11	23.4
24-44 years	14	29.8
45-65 years	15	31.9
66-86 years	7	14.9
Total	47	100.0

3.3 Distributions of Bacterial Infection in Wound Pus, Burn, Medical Device Tips and Blood Specimens Collected from Patients

Overall, Gram-positive organisms *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Strep agalactia* equally and *staph saprophyticus* and *Step*

pyogen equally and accounted for most of the isolates 26 (55.3%). Gram-negative bacteria constituted about 21 (44.6%) of isolates. Among the Gram-negative isolates identified, *Klebsiella* spp (*klebsiella oxycota* and *Klebsiella pneumoniae*) was the predominant isolate, followed by *E. coli* with an isolation rate of 19.1% and 10.6% respectively.

3.4 Frequency of Isolated Pathogens from Each Nosocomial Infection

The wound pus was the most samples from which bacteria were isolated, followed by medical device tip swab, and least isolation was from blood specimen. *klebseilla* spp and *Staph aureus* were the most prevalent pathogens of wound pus contagion respectively. A total of 7 patients with suspected septicemia were studied. The most frequent isolates were gram positive cocci (12.7%). *Pseudomonas aeruginosa* spp 1 (2.1%) was also fairly frequently identified.

3.5 Antibiotic Sensitivity, Resistance and Intermediate Sensitivity of Bacteria Isolated from Wound Pus, Medical Device Tip, Burn and Blood Culture to Septrin and Doxycycline

In the present study, the antimicrobial resistance profile of isolates had been analyzed. Almost isolated strains showed a high ratio of resistance to the Septrin than Doxycycline.

Table 3. Distributions of bacterial infection in wound pus, burn, medical device tips and blood specimens collected from patients

Bacteria	Frequency	Percent
<i>Acinetobacter baumannii</i>	2	4.3
<i>Citrobacter freundii</i>	1	2.1
<i>E. coli</i>	5	10.6
<i>Enterococcus faecalis</i>	2	4.3
<i>Serratia marcescens</i>	1	2.1
<i>Klebseilla pneumoniae</i>	3	6.4
<i>klebseilla oxycota</i>	6	12.8
<i>proteus mirabilis</i>	2	4.3
<i>pseudomonas aeruginosa</i>	1	2.1
<i>staph aureus</i>	10	21.3
<i>Staph epidermidis</i>	3	6.4
<i>staph saprophyticus</i>	1	2.1
<i>Step pyogen</i>	1	2.1
<i>strep pneumoniae</i>	7	14.9
<i>Strep agalactia</i>	2	4.3
Total	47	100.0

Table 4. Frequency of isolated pathogens from each nosocomial infection

Isolates	Specimen	Frequency	Percent
<i>Acinetobacter baumannii</i>	Wound pus	2	4.3
<i>Citrobacter freundii</i>	Wound pus	1	2.1
<i>E. coli</i>	Medical device tips	2	4.3
<i>E. coli</i>	Burn	1	2.1
<i>E. coli</i>	Burn	2	4.3
<i>Enterococcus faecalis</i>	Wound pus	2	4.3
<i>Serratia marcescens</i>	Wound pus	1	2.1
<i>Klebseilla pneumoniae</i>	Medical device tips	2	4.3
<i>Klebseilla pneumoniae</i>	Burn	1	2.1
<i>klebseilla oxycota</i>	Wound pus	5	10.6
<i>Klebseilla oxycota</i>	Wound pus	1	2.1
<i>Proteus mirabilis</i>	Wound pus	2	4.3
<i>pseudomonas aeruginosa</i>	Blood	1	2.1
<i>staph aureus</i>	Blood	2	4.3
<i>staph aureus</i>	Burn	1	2.1
<i>staph aureus</i>	Wound pus	5	10.6
<i>staph aureus</i>	Medical device tips	2	4.3
<i>Staph epidermidis</i>	Blood	2	4.3
<i>staph saprophyticus</i>	Wound pus	1	2.1
<i>Staph epidermidis</i>	Wound pus	1	2.1
<i>Step pyogen</i>	Wound pus	1	2.1
<i>Strep pyogen</i>	Medical device tips	1	2.1
<i>strep pneumonia</i>	Burn	1	2.1
<i>strep pneumonia</i>	Blood	2	4.3
<i>Strep pneumonia</i>	Wound pus	4	8.5
<i>Strep agalactia</i>	Wound pus	1	2.1
Total	47	47	100.0

Table 5. Antibiotic sensitivity, resistance and intermediate sensitivity of bacteria isolated from wound pus, medical device tip, burn and blood culture to Septrin

Susceptibility patterns	SXT		DO	
	Frequency	Percent	Frequency	Percent
R	35	74.5	21	44.7
S	12	25.5	26	55.3
Total	47	100.0	47	100.0

Note: R- Resistant; S-Sensitive

3.6 Resistance Pattern of Isolated Pathogens to Evaluated Antibiotics

All the bacterial isolates were tested for susceptibility against Septrin. Out of 47 bacterial isolates, 12 (25.5%) of the isolates remain susceptible to Septrin. While 35 (74.4%) were found to be resistant to Septrin. The susceptibility forms of the bacterial isolates to Gentamycin antibiotics are offered in Table 7. From the outcomes, 26 (5.3%) isolates were most sensitive to Doxycycline. The rate of resistance

of isolates to Doxycycline antibiotics was 44.6%. The action of Doxycycline against the isolates was somewhat acceptable.

3.7 Susceptibility Pattern of each Nosocomial Infection to Evaluated Antibiotics

Table 7 illustrates the susceptibility character of nosocomial infections to antibiotics. As shown, Wound pus displayed the most sensitivity to Doxycycline antibiotic than antibiotics.

Table 6. Resistance pattern of isolated pathogens to evaluated antibiotics

Isolates	SXT susceptibility		Total	Do susceptibility		Total
	R	S		R	S	
<i>Acinetobacter baumannii</i>	2	0	2	1	1	2
<i>Citrobacter freundii</i>	0	1	1	0	1	1
<i>E. Coli</i>	4	1	5	1	4	5
<i>Enterococcus faecalis</i>	2	0	2	1	1	2
<i>Serratia marcescens</i>	1	0	1	0	1	1
<i>Klebseilla pneumoniae</i>	1	2	3	2	1	3
<i>Staph epidermidis</i>	1	2	3	1	2	3
<i>Step pyogen</i>	1	0	1	0	1	1
<i>Strep agalactia</i>	2	0	2	2	0	2
<i>klebseilla oxycota</i>	5	1	6	3	3	6
<i>proteus mirabilis</i>	2	0	2	1	1	2
<i>pseudomonas aeruginosa</i>	1	0	1	0	1	1
<i>staph aureus</i>	7	3	10	5	5	10
<i>staph saprophyticus</i>	1	0	1	0	1	1
<i>strep pneumonia</i>	5	2	7	4	3	7
Total	35 (74.4%)	12 (25.5%)	47	21 (44.65)	26 (5.3%)	47

Table 7. Susceptibility pattern of each nosocomial infection to evaluated antibiotics

Sample	SXT Susceptibility		Total	DO Susceptibility		Total
	R	S		R	S	
Blood	4	3	7	1	6	7
Wound pus	25	4	29	14	15	29
Medical Device Tip	3	4	7	5	2	7
Burn	3	1	4	1	3	7
Total	35	12	47	21	26	47

3.8 Comparison of Sensitivity Patterns to Septrin, Doxycycline Antibiotics and Age Groups of Patients

Patients between 3 to 65 years old (n=30) had a somewhat high resistance to Septrin. In the age group 66 to 86 years (n=5), the resistance to septrin is somewhat reduced.

3.9 Distribution of Isolates by Ages

In females, the most predominant wound and blood stream infection was *Staph aureus* among 45-65. While *Strep pneumonia* is considered as

the second type isolated from the age group of 24-44.

3.10 Prevalence of Different Bacterial Growth among Female and Male Patients

A total of 47 specimen culture results of patients with suspected infection during twelve months period were studied. The isolates and sex distribution of the patients are shown in Table 9. Bacteria isolation rate was in females 25 (53.1%) than males 22 (46.8%) had a positive bacterial culture result. *Staph aureus* was the most frequent isolate from female patients.

Table 8. Comparison of sensitivity patterns to Septrin, Doxycycline antibiotic and age groups of patients

Age	SXT susceptibility		Total	DO susceptibility		Total
	R	S		R	S	
3-23	10	1	11	6	5	11
24-44	10	4	14	4	10	14
45-65	10	5	15	9	6	15
66-86	5	2	7	2	5	7
Total	35	12	47	21	26	47

Table 9. Distributions of isolates by ages

Isolates	Age				Total
	3-23	24-44	45-65	66-86	
<i>Acinetobacter baumannii</i>	1	0	1	0	2
<i>Citrobacter freundii</i>	0	0	1	0	1
<i>E. Coli</i>	1	3	0	1	5
<i>Enterococcus faecalis</i>	0	2	0	0	2
<i>Serratia marcescens</i>	1	0	0	0	1
<i>Klebseilla pneumoniae</i>	0	0	1	2	3
<i>Staph epidermidis</i>	0	3	0	0	3
<i>Step pyogen</i>	0	0	1	0	1
<i>Strep agalactia</i>	1	0	1	0	2
<i>klebseilla oxycota</i>	3	2	1	0	6
<i>proteus mirabilis</i>	1	0	1	0	2
<i>pseudomonas aeruginosa</i>	0	0	0	1	1
<i>staph aureus</i>	1	1	6	2	10
<i>staph saprophyticus</i>	0	0	1	0	1
<i>strep pneumonia</i>	2	3	1	1	7
Total	11	14	15	7	47

Table 10. Prevalence of different bacterial growth among female and male patients

Bacteria	Sex		Total
	Female	Male	
<i>Acinetobacter baumannii</i>	1	1	2
<i>Citrobacter freundii</i>	0	1	1
<i>E. coli</i>	3	2	5
<i>Enterococcus faecalis</i>	2	0	2
<i>Serratia marcescens</i>	1	0	1
<i>Klebseilla pneumoniae</i>	2	1	3
<i>Staph epidermidis</i>	1	2	3
<i>Step pyogen</i>	1	0	1
<i>Strep agalactia</i>	1	1	2
<i>klebseilla oxycota</i>	4	2	6
<i>Proteus mirabilis</i>	0	2	2
<i>pseudomonas aeruginosa</i>	0	1	1
<i>staph aureus</i>	6	4	10
<i>staph saprophyticus</i>	0	1	1
<i>strep pneumonia</i>	3	4	7
Total	25	22	47

Table 11. Distributions of susceptibility patterns of isolates to septrin, doxycycline by sex

Gender	SXT			DO		Total
	R	S	Total	R	S	
Female	21	4	25	11	14	25
Male	14	8	22	10	12	22
Total	35	12	47	21	26	47

3.11 Distribution of Susceptibility Patterns of Isolates to Septrin, Doxycycline by Sex

In this study, a total of 47 isolates from diverse swabs and blood samples were experimented

with *In vitro* by the disk diffusion test to define the susceptibility of these bacteria to Septrin. Isolates that were resistant to Septrin were shown in females more than males. Isolates that were resistant to Doxycycline was also shown in females more than males.

4. DISCUSSION

In the present research, *Staph aureus* was the most frequently isolated pathogen in our study followed by strep pneumonia and *klebsiella oxycota*. This is agreeing with the statements of several studies that expressed *S. aureus* as the most familiar bacteria rescued from wound infection [17,18].

Bloodstream infection is a demanding issue, and occasionally, it may be life-threatening; consequently, timely detection, identification, and antimicrobial susceptibility testing of blood-borne. Culture positivity was noticed in 7 (14.8%) of adult patients. In this study, males (n=6) had high culture positivity with septicemia as compared with females (n=1).

The result was consistent with the investigations done by Hussein et al. [19]; Kaur and Singh, [20] who registered high culture positivity in men. The maximum number of positive blood cultures was from age of 75, and the minimum was from the age of 27 years.

Gram-positive cocci were the most ordinary organism isolated. These results studies are analogous to a study done by Kokku et al. [21], but not consistent with Henry et al. 2018, were noticing Enterobacteriaceae was the most commonly isolated gram-negative organisms, constituting 67% (122/184) of all gram-negative bacilli [22].

Several studies have been accomplished to determine the sources of bacterial infections in hospitals. Clearly, bacterial colonies have been recognised at various hospital environment sites including beddings, stethoscopes [9], computers, catheters [12] and other small electronic devices and instruments utilized by health workers.

There was 7 medical equipment that had bacterial isolates determined from the sample in this study. Of the bacteria found, the most frequent isolates were *E. Coli*, *Klebsiella pneumonia*, *staph aureus* and *strep pyogen 2* (4.2%) equally. A case study by Ameh et al., investigating the prevalence of bacterial isolates on hospital surfaces in Nigeria [23] found the following isolates with prevalence: *E. coli* (34.4%), *Klebsiella* species. (21.9%), *Pseudomonas* species. (15.6%), *Staphylococcus* species. (12.5%), *Proteus* species (9.4%).

Most of the world literature indicates that *staph aureus* is one of the main pathogens accountable

for a number of infections in hospital settings, with considerable morbidity and mortality [24].

S. aureus represents one of the most severe gram-positive bacterial infections in nosocomial and community settings. The severity of *S. aureus* infections is related to the different potential infected tissues (ranging from skin to bloodstream). *S. aureus* strains were recovered from various materials and analyzed with traditional microbiological tests to specify their antimicrobial susceptibility. Hospitalized patients show a high frequency of *S. aureus* infections due to their weak immune system and frequent injections and catheterizations [25,26]. Moreover, in these types of patients, *S. aureus* can lead to life-threatening infections such as endocarditis and osteomyelitis. Our study indicates a high rate of resistance of isolates to Septrin than Doxycycline. Analogous to our results, in the study of Sadeghi et al. [27].

5. CONCLUSION

In conclusion, the problem of antibiotic resistance is very serious in Libya and appears to be on the rise. In this study, the prevalence of antibiotic resistance was high in most common pathogenic strains. Therefore, the results of this study demonstrated that both antibiotics with a high resistance level must be less used for the treatment of infections. Moreover, to prevent the spread of resistance among various strains and improve the effectiveness of antibiotics, it is suggested to establish a precise schedule for antibiotic use in this region based on their antibiotic resistance pattern. The health authorities in particular and society in general should address the problem of antimicrobial resistance urgently. Establishing monitoring systems based on routine testing of antimicrobial sensitivity and education of healthcare workers, pharmacists, and the community on the health risks associated with the problem and benefits of prudent use of antimicrobials are some of the steps that can be taken to tackle the problem in the future.

6. LIMITATION OF THE STUDY

The study did not determine the resistance detected was due to hospital-acquired or community acquired infection, also the study did not show the trends of antibiotics resistance from year to year.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

The study was approved by the Ethics Committee of Al Saleem Medical Laboratory.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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