Al-Kufa University Journal for Biology / VOL.10 / NO.2 / Year: 2018
Print ISSN: 2073-8854 & Online ISSN: 2311-6544

Isolation and Identification of Bacterial Wound Infection Isolates and their Antibiotic Susceptibility Pattern

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Abstract

Wound Infections are continuous to be a major complication with significant increase in costs, morbidity and potential mortality. This study was aim to screening the bacteriological observation on wound infection in both inpatients and outpatients, the effective antibiotic and serotyping for the prevalent bacteria. The study was carried out on two hundred patients of different age groups to isolate the causative organisms encountered in wound infection and their antibiotics. The percentage of wound infection among outpatients was found to be 84%, similarly the percentage of wound contamination inside the hospital (Medical City Teaching Hospital) also was found to be 84%. The relation between sex and wound infection was studied, no effect of sex on the rate of infection was found.A total of 152 bacterial isolates were predominant *Staphylococcus aureus* (76, 50%), followed by *Escherichia coli* (36, 23.69%), Beta-haemolytic streptococci(13,8.55%),*Klebsiella aerogenes* (8,5.26%), *Pseudomonas aeruginosa*(7,4.6%), *Proteus* spp.(7,4.6%) and *Streptococcus faecalis*(5,3.29%).*S.aureus*was the most common among outpatients(57%), bacteriophage-typing of this bacteria was made on the total number (76 isolates)from both inpatient and outpatients, thirty-two strains found to be typable, while the remaining (44 strains) were not typable; no predominance of a particular phage-group was observed. *E. coli* was the common bacteria among inpatients (28%), the rate of fecal carriage were 100%. The antibiotics of both, fecal and wound of the same patient were similar. Beta-haemolytic streptococci isolated at a rate of 6.5% and group A was the most prevalent group in wound infection. Regarding the antibiotic susceptibility, superiority of resistance to many antibiotics in treating *Pseudomonas aeruginosa*, *Proteus* spp., *E.coli*, *S. aureus* and *S. faecalis* was observed. Most wound Infections were polymicrobial and predominant isolate was *S. aureus.* Isolates showed high level of resistance to antibiotics, which invites us to panic and anxiety.

Keywords: Wound Infections; bacteria, polymicrobial, *Staphylococcus aureus*, susceptibility.

Introduction

Wound infection have been defined as wound with pus observable to the eye, whether or not microorganisms may have been cultured from the infected substance. In some cases in which no wound culture was done and the criteria of pus and clinical signs of infection were satisfied such cases have been regarded as infection[1].They are one of the majority common hospital acquired infections and are an important cause of morbidity and mortality. Progress of such infections represent belated healing, cause anxiety and worry for patient, longer stays at hospitals and add to cost of healthcare services significantly[2,3]. Different microorganisms colonize wound and in some patients one or more species of organisms proliferate in the wound, which may lead to tissue damage, host response accompanied by inflammation, that is, clinical infection [4].

All wounds are contaminated by both pathogens and body commensals ranging from bacteria and fungi to other parasites, but Bacteria and viruses cause most diseases andin many cases there is a mixed infection with more than one bacterial species [5,6]. Bacteriological studies have also revealed that wound infection is worldwide and that the bacterial kinds present differ with geographical site, bacteria dweller on the body skin, clothing at the place of wound, time between wounds and examination [7,8]. In healthy individuals, the infection is usually only superficial, and local treatment with a spray containing one of the combination of antibiotics is recommended. If deep-seated infection develops, systemic treatment may be necessary, in which case the choice of drug will depend on the sensitivity of the infecting organism(s)[7,9]. Hence, both gram positive and gram negative bacteria are caused infection, such wounds may become infected by one or more bacterial spp**.,** of which, the most frequent are, *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Proteus spp*. and *Pseudomonas aeruginosa*. Hospital wound infections are remain an significant trouble in hospital care and various efforts have been ready to verify the most vital spreading ways and to find the mainly useful prophylactic measures [5,10,11].The routine use of antibiotics in both hospitals and clinics has resulted in wide spread antibiotic resistance. Thus the handling of wound infection are requisite to assess the exact type of antibiotics and the suitable attentions to be used in infections, taking into thought the etiology of the infection and the time of the antibiotic treatment [12].

This study was aim to screening the bacteriological observation on wound infection in both hospitalized and non-hospitalized patients, also the effective antibiotic and serotyping for the prevalent bacteria.

Patients and Methods

This study was conducted in the Medical City Teaching Hospital in Baghdad – Iraq, over a period of one year (January to December 2015). Wound swabs were obtained from two hundred patients, one hundred were non hospitalized patients and they were chosen randomly as outpatients, their age ranged between 10 days -60 years. While the other, were hospitalized patients (inpatients).Those patients were divided into neonatal; diabetic and others patients were further subdivided into the following: patients with clean wounds;clean-contaminated wounds and contaminated wounds.

Nasal swabs were taken from thirty surgeon and nurses for epidemiological purpose. General information such as name, age, sex, residence, occupation and whether antibiotic has been received, were taken from each patient before obtaining the swab.

Specimens:

Disposable sterile swabs were used; the specimens were obtained by rolling the swab over the wounds. Swabs were immediately carried to the lab, and inoculated onto Blood, MacConkeys and nutrient agar plates, incubated at 37 ^{o}C for 24-48 hours aerobic condition. Bacteriological culture and examination was done following standard microbiological techniques [13,14].

Identification of Streptococcus groups

Lancefield serotyping was performed using a Streptex kit (Wellcome Diagnostics, Dartford, England). Sensitized latex suspension (antisera A,B,C,D,F and G.)were used through a slide method for the rapid identification of Lancefield groups A,B,C,D,F and G of beta-heamolytic setreptococci. Pure culture of beta-heamolytic streptococci was made on fresh blood agar plates. A dense suspension was made from the above culture in 0.4 ml of the extracting enzyme solution, mixed in a test tube and placed in a water bath at 56^{o}C for 1 hour. The tube was then centrifuged for 10 minutes; the supernatant, which contains the extracted antigen for streptococcal grouping, was then collected. Six drops of the above extract (supernatant) were placed separately on a clean slids, to each a drop of each of the six latex suspension (anti A,B,C,D,F and G) were added and mixed by using applicator sticks, rotated and then the mixtures were observed after 2 minutes for the presence or absence of visible agglutination[15,16].

Antibiotic susceptibility testing:

The determination of bacterial susceptibility of different isolates to various antibacterial agents in this study was made by using Kirby Bauer method: Aloopful of the infusion broth containing the tested organism was inoculated into about 10 ml. of sterile, cooled, melted nutrient agar. Immediately the content of this tube was poured homogenously onto the surface of a Mueller Hinton agar plate according to Clinical and Laboratory Standard Institute (CLSI,

2012) guidelines. Ampicillin(AP-10µg),Trimethoprim-sulfamethoxazole (TMP/SMX-1.25/23.75µg), Cefotaxime (Cf-30µg), chloramphenicol (C-30µg), Kanamycin(K-30µg), Gentamycin(GM-10µg), Sulfonamides(ST-250 µg),Norfloxacin(N-10µg), Rifampicin(RP-10µg), Carbincillin(CB-100µg), Tetracycline(Te-30µg), Streptomycin(S-10µg), Cloxacillin(cx-1µg), Erythromycin(E-15µg), Penicillin(PG-10 units), Linezolid(L-30µg), Nitrofurantoin(F/M-300µg), Colistin(Co-10µg), Tobramycin(Tm-10µg), (Hi Media, India). Discs were generally applied onto the agar surface using an 8 or 12 cartridge dispenser, plates were incubated at 37^{o}C for 18-24 hours. Sensitivity of an organism to an antibiotic is demonstrated by a zone of inhibition around the antibiotic disc. The *E. coli* ATCC 25922 strain was assumed as the standard for quality control [17].

Statistical analysis;

Differences between proportions were assessed by Chi-square analysis.

Results

In the present study, out of the total 200 samples, 168(84%) showed significant bacterial growth and 32(16%) with no growth. Samples were divided in two groups, the first group (hundreds of outpatients) 84 patients showed positive cultures (growth of pathogenic bacteria), the remaining 16 cases proved negative (no growth of pathogen).The second group (hundreds of inpatients)84 patient showed positive cultures while 16 proved negative(figure 1, table 1).

Only three swabs (nasal) from the hospital staff yielded α-haemolytic streptococci while the remaining swabs (27) yield growth of *S. epidiermidis* only. A total of 168positive cultures152(90.4%)yielded significant bacterial growth indicative of pure cultures and16(9.5%) were mixed. Among the positive specimens of the outpatients,80 (95.2%) yielded pure cultures of single organism, 4(4.8%) were mixed cultures, while the positive specimens of the inpatients showed 72(85.7%) pure cultures of a single organism and 12(14.3%) were mixed cultures (table 1,figure 2).

The incidence of Gram-positive bacteria among outpatients was 67(80%), which is much more than the Gram-negative bacteria 13(15%). In the inpatients, the incidence of Gram-positive bacteria was 27(32%), which is less than the Gram-negative bacteria 45(54%) as shown in table1, figure 3, A and B. The most common Gram positive organism isolated especially among the outpatient swas *S.aureus*(80.8%)as shown in table II. The total number of the isolates was seventy-six from both inpatients and outpatients. Among the outpatients, was fifty-seven (67.9%), while for the inpatients was lesser 19(22.6%)(figure2). *S.aureus* phage typing was carried on, for both hospitalized and non-hospitalized patients. Thirty member of the hospital staff were obtain nasal swabs. All nasal swabs showed growth of normal flora and non had shown growth of *S.aureus*. *E.coli* was common Gram negative bacteria found among inpatients and outpatients 36 (62%)(tableII), it was commoner among inpatients (28%) than outpatients(8%). Cultures and antibiotic sensitive for both, the stool and the swab obtained from the wound of the same patient was done to give an idea about the autogenic infection in hospital. The total number of Gram positive β- haemolytic streptococci among patients was thirteen (13.9%); it was more frequent among outpatient 9 (10.4%) than inpatients 4 (4.76%),most of the isolates were belonged to group A(9 cases).*K.aerogenes* was eight isolates(13.8%), and it was commoner among inpatients 6 (7.1%) than outpatients 2 (2.4%). Seven isolates (12.1%) were found of *P. aeruginosa* during the present work and it was commoner among inpatients 6(7.1%) than outpatients 1(1.19%). In the same table, seven (12.1%) cases of *Proteus* species were recorded, most of them 5(6%) among inpatients and the remaining 2 (2.4%)were among outpatients. The total number of *S.faecalis* was five (5%) cases, four (4%) of them among inpatients and one (1.19%) recorded among outpatients (table II, figure 2). As it is indicated in table III, the relationship between age distribution and the most common isolated pathogens from wounds were Staphylococcal infection more frequent in the adult age (25-44 years) than other ages while infection of *E.coli* was more frequent among inpatients especially at old age (45-64 years).Data from table IV showed that high infection rate (39.3%) occurred at old ages.

In examining the sensitivities of different organisms isolated from wounds in this study to nineteen various antibiotics, it was obvious from results, that *S.aureus* was fully resistant 76(100%) to three antibiotics;Kanamycin, Norfloxacin, Carbincillin and high resistance range(70-90%) to Ampicillin, Trimethoprim-sulfamethoxazole, Tetracycline, Streptomycin, Cloxacillin, Erythromycin, Penicillin, Linezolid and moderate susceptible to Cefotaxime (64.4%),chloramphenicol (68.4%), Gentamycin (68.4%) and Rifampicin (67.1%) (figure 4a). *E.coli* similarly was found to be high resistant range (70-97%) to Colistin, Nitrofurantoin, Tetracycline, Ampicillin, Streptomycin, Sulfonamides, Norfloxacin, Carbincillin, Kanamycin, Tobramycin, chloramphenicol, Trimethoprim-sulfamethoxazole, Rifampicin. Moderate susceptible 66.6% to Gentamycin and 69.4 % to Cefotaxime (figure 4b). *P. aeruginosa* showed entirely resistant (100%) to five antibiotics; Ampicillin, Trimethoprim-sulfamethoxazole, Cefotaxime, Norfloxacin, Colistin (figure 4c).Similarly *Proteus* and *K. aerogenes* showed high resistance rate(71- 87%) to all antibiotics in this study(figure 4d,4e). However, *S. faecalis* was resistant to all antibioticsin this study while in a lesser degree (60%) to both Cefotaxime and Gentamycin (figure 4f).

Infected diabetic wounds and antibiotics:

Ten out of hundred hospitalized patients(10%)were apparently diabetic and had wound infections, some of the wounds yielded a mixture of more than one bacteria, while others yielded only pure culture of one bacteria. The most prevalent bacteria in their wounds were *E.coli* (30%) and *Proteus* spp.(20%),while *S.faecalis*, *P.aeruginosa* and Beta-haemolytic Streptococci, *S.aureus* and *K.aerogenes*,were (10%) for each one. The antibiotic of the above-mentioned organisms, which were obtained from the diabetic wounds, showed that Gentamycin and Cefotaximewas the most effective antibiotic against them (100% a susceptibility).

Antibiotic of neonatal wound infections:

Some of the swabs (16.7%) obtained from the infected umbilical cord of new born babies(1-10 days) yielded a mixture of tow organisms , while other 5(83.3%)yielded a pure culture of one bacteria. The most common organisms were *S.aureus* 4(66.6%), while *E.coli* 1(16.7%)and *S.faecalis*1(16.7%). The antibiotic of the above causative bacteria showed, that they were susceptible to Gentamycin (100%) and Cefotaxime (90%).

Antibiotic sensitivity of Beta-haemolytic Streptococci:

The antibiotic sensitivity test of β-haemolytic Streptococci was carried out separately on blood agar media. The results showed in Table V. that it is susceptibility to antibiotics were as Erythromycin 100%, Ampicillin 84.6%., Penicillin 77%, Chloramphenicol 61.5%, Tetracycline 53.9%,Cefotaxime 46.1%,Streptomycin 46.1% and Cloxacillin 23%. The total number of β-haemolytic streptococci isolated from inpatients and outpatients was thirteen; However, nine of these isolates were belonged to group A β- haemolytic Streptococci, and the remaining four isolates did not belong to any one of the other groups tested B,C,D,F and G by using strepex test.

Phage-typing of S.aureus: Seventy-six strains of *S.aureus* (Coagulase positive) were isolated from different infected wounds. The phage-typing technique carried out in this study to know the different phage-types and phage-groups, showed that twenty different phage-types were found out of thirty-two typable strains(42.10%),and the remaining fourty-four strains were non-typable(57.89%)organism as shown in table VI. However, the most common phage-types noted in the present work were 3C and 29, of groups II and I respectively, as it is indicated in table VI. The data in table VII showed that *S.aureus* phage 3c were universally sensitive to rifampicin and cefotaxim with a 100% sensitivity to these two agents.

Discussion

Wound Infection was a major problem in the face of surgical and medical progress throughout the entire history of surgery and medicine. For many centuries of years it was the signal most important medical obstacle. thought that wounds infection, and patients, maybe made high susceptible to infection has been documented for about five decades. So has received most interest in our hospitals.

The present work was carried in the Medical City Teaching Hospital in Baghdad – Iraq, on two hundred patients with wound infection over a period of one year (January to December 2015). One hundred were non –hospitalized patients, while the other hundred patients were hospitalized patients.

The bacteriological data following culturing the swabs obtained from the wounds of the above two hundred showed that, ninety four have had a Gram positive bacteria, fifty eight had a Gram negative bacteria and sixteen patients yielded a mixed culture of different bacteria, while the remaining patients (thirty two), their wound cultures showed no growth (negative cultures, so they were considered as negative cases) (figure1, 2).The above mentioned finding may are agreement with what has been reported by Opalekunde *et al*.[18] and Mohammed *et al*.[19]. The reason for negative cultures may be attributed to the misuse of antibiotics or the inefficient anaerobic technique, play role in this.

Among the outpatients *S.aureus* was the chief frequent organism isolated (57%) as shown in table I, this may be due to the possibility that non-hospitalized patients, while the percentage of *S.auerus* among inpatients was less than those of outpatient (19%),this low percentage (table I, figure 2)as expected might well be due to the daily misuse of antibiotic in the hospital without prior consolation of the bacteriological laboratory, and possibly to the efficient procedures of cleansing of wounds. Nearly all of the wound infection in these health cases were poly-microbic in environment and in main cases, related with *S. aureus* and many other bacteria and microorganisms. Finding may similar to study by Verma [5] and Rao *et al.*[20]. Revealed a different types of bacteria from wound but *S.aureus* was the predominant microorganism.

Regarding *E.coli* infections, the percentage was less among outpatients(8%) than inpatients (28%)as shown in table I and figure 2, however as mentioned earlier, such results are not unexpected, since the wide use of antibiotics results in the survival of the resistant organisms such as *E.coli* and reduction of those which are sensitive [21,22]. It is of interest to note, that the antibiotic of *E.coli* isolated from wound of inpatients was identical with that of the *E.coli* isolated from the stool sample of the same patient.

A finding which could support the believe of an endogenous infection, this can be further studied and confirmed by comparing the serotypes of these different isolates (*E.coli* from wound and stool of the same inpatients).

Beta- haemolytic streptococci was other bacterial pathogen isolated in this study, thirteen out of the total two hundred (6.5%) collected (9% from the inpateint 4% from outpatients). This indicates that this organism is less common than *S. aureus* and *E.coli* in wound infections, though the total number of patients in this work is moderate, but the lessRao *et al.*,[20] have collected a comparable number of cases (two hundred)and the beta-haemolytic streptococci were isolated at a rate of 11.23%only. The streptococcal grouping carried on the thirteen isolates, showed that group A is the most common (9 isolates) while the other four isolates did not belong to group A, or any of the groups which are commonly known (B, C,D,E,F and G); However this predominance of group A beta-haemolytic streptococci is expected [23].

Anaerobic streptococci and other anaerobic bacteria in general (*Clostridial spp*.) were not isolated in the present work, this could be explained on the basis of two possible reasons, modern anaerobic isolation techniques were not used throughout the whole work, because they were not available, and the other reasons was the majority of the patients suspected of having gas-gangrene were usually discharged to Homiat-Hospital, before specimens and information were being drawn from them.

P.aeruginosa was isolated from infected wounds (table I, figure 2) of both outpatient and inpatient, however the rate of isolation of this organism among the inpatient was higher (6%) compared to that of outpatients (1%), though this is expected, as shown in a recent study done in Africa, India and Pakistan [20,22,24,25] to assess the role of *P.aeruginosa* in different types of wound infection; but still, the low percentage of pseudomonal infections in the hospital environment was encouraging and could possibly indicates, to Gram positive coccai staphylococcal infection to be the predominant microorganism (table II). That reason may illustrate low contamination with this organism during operation, post-operative wound, floor and toilets [10,21,22,26]. The other organisms isolated in this study were *K.aerogenes* (4%), *Proteus Spp*.(3.5%) and *S.faecalis* (2.5%), those organisms were common among outpatients, and the results are the near to those reported by[6]. Hospital infections continues to be an important and serious problem in hospital health care. Intensive attempts have been made to identify the most important methods of transport of bacteria and to find the best effective preventive measures for their disposal. From the present survey the staff seemed to present no special hazard of transferring infections and this will decrease the risk of cross-infection in the hospital, since the hospital staff are usually considered as an important source of infection in hospital as in the survey done by [8,27]. Wound infection is commoner after certain operations, and as operations vary in frequency in different units and with time, it is necessary to have a classification for the type of incision (clean, clean-contaminated and contaminated)[28,29].
In this arrangement, the organisms isolated from wounds after clean operations were as follows: *S.aureus*(6%), *E.coli*(5%), *P.aeruginosa*(2%), *K.aerogenosa*(1%), *Proteus spp*.(1%), *S.faecalis* (1%), mixture of two organisms(1%) and no growth of pathogenic organism (negative culture) (2%). The above results indicate that *S.aureus* was common in clean operations, however this finding is in agreement with what was shown in an earlier study [9,20], while the percentages of different organisms isolated after clean-contaminated operations were: *S.aureus* (9%), *E.coli* (6%), mixture of more than one organism (4%),*K.aerogenes* (3%), *Proteus spp.(*3%) *P.aeruginosa* (2%), no growth of pathogenic organism (negative culture) (8%), those percentages were differed from percentages of organisms, isolated after contaminated operations which were: *E.coli* (12%), *K.aerogenes* (3%), *S.aureus* (3%), mixture of more than one organism (3%), *Proteus spp*.(2%),*P.aeruginosa* (1%), *S.faecalis* (1%), no growth of pathogen (negative culture) (5%). As could be expected, intestinal bacteria were common in clean-contaminated and contaminated operations, since these wound classes are involved in operations of the colon, rectum, stomach and gall bladder, where intestinal flora are then expected.

The finding of, Nazeer *et al*.[24] and Raza *et al*.[30] in their study on clean contaminated and contaminated wound infections were nearly similar to those findings of the present work and the variation might well be due to the large number of cases they have investigated. *S.aureus* was isolated as shown earlier after clean-contaminated operations (9%),though their number is unexpected, because intestinal flora were the ones expected, it could be due either to direct or indirect contaminations[31]. However, further study and assessment are required. Ratemo [32] and Vieira *et al*.[33] were carried out a study on infections that are generally encountered among neonates, they found that *S.aureus* was the most predominating pathogen isolated from the umbilicus, this finding is in agreement with what had been observed in the present work . The possible reason for difference in these studies could be recognized to differences in the populations investigated, variety of surgical procedures completed on the study participants, also timing of specimen collections.

Antimicrobial Susceptibility Pattern:

Recent advances in medical technology and therapeutics(especially antibiotics)are producing greater number of highly susceptible patients, and this aggravated by the occurrence of transferable resistance to antibiotics in pathogenic organisms [32,27]. The objective of studying or assessing the antibiotics in this study of different pathogens isolated from infected wounds, was to provide physicians and surgeons with possible drugs of choice to treat infected wounds prior receiving the results of cultures and antibiotic sensitivity tests, so that it will save time, and results in a minimizing patient complains [45]. In the present study the greater part of the isolates were gained from patients who were previously on antibiotic treatment, and this could have led to the short recovery of antibiotic susceptible pathogens. May that's led to superiority resistance to all antibiotics in treating *S. aureus, S. faecalis, P.aeruginosa, Proteusssp, K. aerogenes* and *E.coli*, as shown earlier (figure 4.a,b,c,d,e,f).This finding may relative to extensive and badly chosen of antibiotics has been shown to increase the progress of antimicrobial resistant bacteria. The sensitivity pattern of Gram positive, is changing due to growing emergence of antibiotics resistant bacteria strains like *S. aureus* and *S. faecalis* making the choice of empirical treatment more difficult and expensive [34,35]. *S. aureus* has been reported to naturally resist this antibiotic [10]. Thus, *S. aureus* have been reported in America, Europe and recently in Africa. Later on cases were reported in hospital environments remarkably among the community without contact to hospitals [6, 31]. *S. aureus* and *S. faecalis* contain several methods of resistance like mutation of porine proteins extra or deposition of high fat coat on cell wall and impermeability of the membrane [36]. Alternatively, may the presence of plasmid mediated β-lactamase producers [32]. Gram negative isolates in this study showed a high antimicrobial resistance. Most resistance of *E.coli, K. aerogenes,P.aeruginosa* and *Proteus ssp.* isolates was exposed to Colistin, Nitrofurantoin, Tetracycline, Ampicillin, Streptomycin, Sulfonamides, Norfloxacin, Carbincillin, Kanamycin, Tobramycin, chloramphenicol, Trimethoprim-sulfamethoxazole, Rifampicin. Multiple drug resistant isolates has been also reported in other studies [37,38,30].Resistant in Gram negative bacterial infections resemble a great challenge in the selection of correct antibiotic may leading to treatment failure, prolonged duration of disease and great danger of death. They have ability of undergoing mutation or acquiring a resistance gene when antimicrobial agents are inappropriately used [38].A possible reason for the high resistance could be due to occurrence of ESBLs production in these bacteria and enlarged unsuitable prescription of antibiotic for treatment or prophylaxis at this hospital [39]. The unwise use of these drugs in the study population leading to high selection pressure of resistant bacteria. Table VI showed clearly that all members of phage-type 3c(6 isolated)were universally sensitive to rifampicin and Cefotaxime, however no information available to compare with these findings. Therefore it is quite obvious from the above findings that gentamycin and Cefotaxime could be well administrated pre-operatively, and also in cases of wound infections prior waiting for the results of culture and sensitivity. Gentamycin could also solve some of the serious consequences that are arise in postoperative wound infections, a problem which is still ubiquitous [40,41]. The resistance of bacteria to antibiotics as used in my opinion most probably to be due to: The misuse of antibiotics by Iraq patients, increased the resistance to those antibiotics, thus newer antibiotics are more efficient than older ones. Cross infections in hospitals also play an important role in resistance of microorganisms to antibiotics, whether patients took the infection in direct or indirect way. The importance of airborne transmission as one of the serious sources of infection in operative theaters remains a clearly exciting problem, but there is strong evidence that could help support the proposal that airborne bacteria can cause wound infections after surgery of wound infections [20,29].

***Staphylococcus aureus* phage-typing:**

The absence of a suitable method for the serological typing of *S.aureus* were classified or typed according to their susceptibility to a set of anti-staphylococcal phages chosen to make as many valid distinctions as possible between staphylococcal strains. It is therefore a method of bacterial classification based on a singles class of character. In the present work phage-typing was used for epidemiological purpose to find out the correlation between the *S. aureus* carriers and the incidence of wound infections. The phage-typing used in the percent work to find the strain(s)of *S.aureus* prevalent at the Medical City Teaching Hospital. Results showed that only thirty two isolates of *S.aureus* were typable which comprises six of the inpatient isolates (31.57%)and twenty six of the outpatient isolates (45.61%). Table VII showed that the most common phage-types encountered among the *S.aureus* were 3C(18.75%),29(12.50%)and 3C/55(9.40%).No correlation could be made, since there is no previous work published regarding phage-typing of staphylococci isolated from the infected wounds in Iraq. Other phage-types observed in this study were 29/3a(3.10%), 29/52(3.10%),52/3a(3.10%), 52/80/81/96(3.10%),52(3.10%),3C/55/71(3.10%),52/80/81(3.10%),29/3C(3.10%),3A/3c/55/71 (6.20%),29/3A/3C(3.10%),71/96(3.10%),42E/54(3.10%),80(3.10%),94/96(3.10%),71(3.10%), 29/3A/3C/71(3.10%) and 79(6.20%). Regarding the phage –groups, no predominance of a particular phage-group was observed, though as expected all the *S.aureus* isolated here fall among phage-group I,II and III(table VIII),therefore the data of the typing did not show a particular strain(s) of *S.aureus* of importance, since there is a known phage-type (47/53/75/77) which is usually encountered in hospital environment [42,43]. The absence of this phage-type and predominating one in the present work could possibly by due either to strain differentiation or to the small number of staphylococcal isolates (76 isolates), therefore the phage-typing of *S.aureus* particularly in the hospital environment, need further investigation and elaborate study. The non typability of *S.aureus*(44 isolates)may be due to blocking of the agglutinations by other antigens or substances, to self-agglutination, or to the strain not being in possession of any of the agglutinations represented in the typing set[43,44].

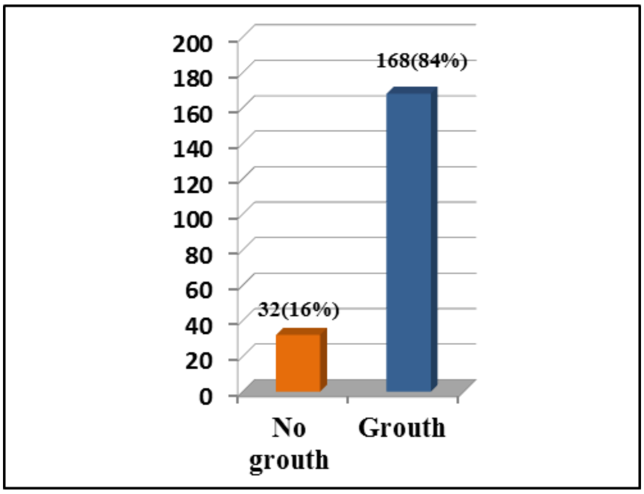


Figure 1: Bacterial growth pattern of the isolates(N=200)

Table I. Distribution of the causative organisms among the inpatients and outpatients at the Medical City teaching Hospital.

Organism	No of cases (outpatients)	No of cases (inpatients)	Total
S.aureus	57	19	76
E.coli	8	28	36
Proteus	2	5	7
Beta-haem.Strep.	9	4	13
Klebsiella	2	6	8
S.faecalis	1	4	5
P.aeruginosa	1	6	7
Mixture	4	12	16
No growth (Negative)	16	16	32
Total	100	100	200

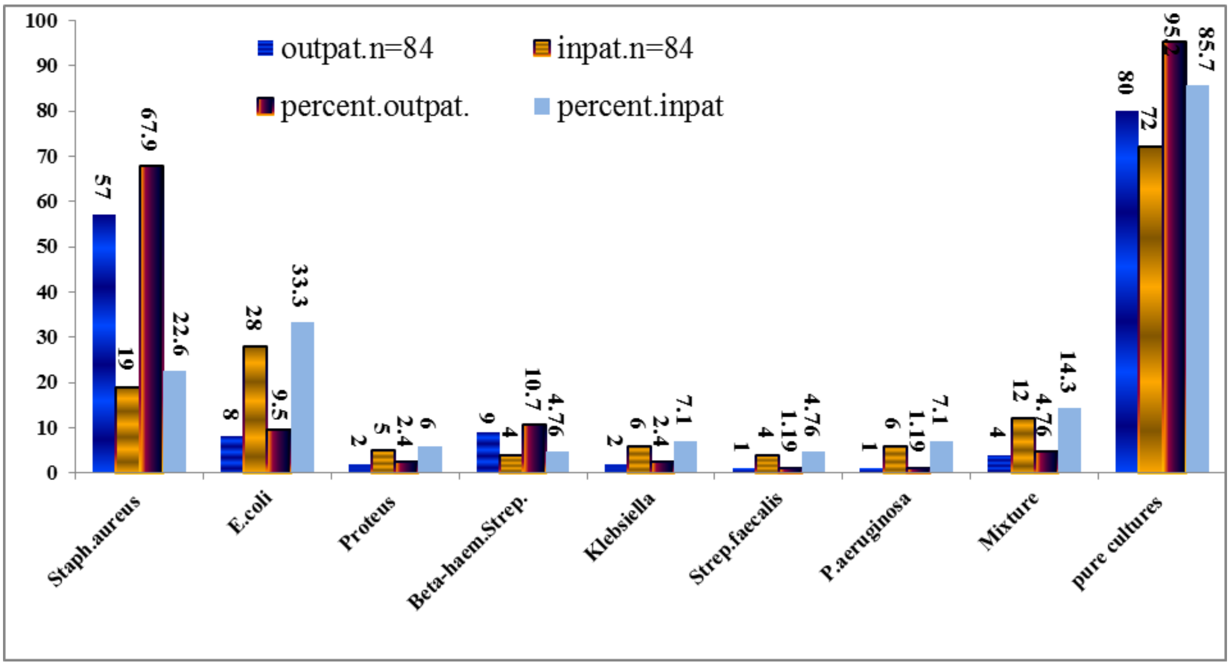
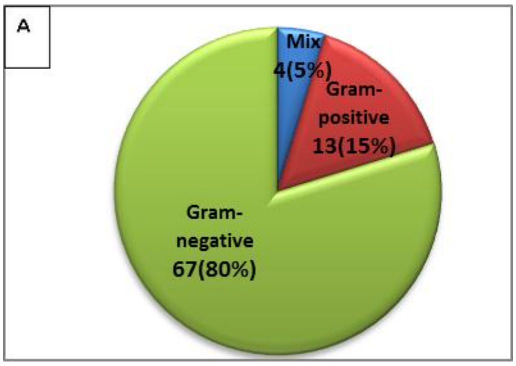


Figure2.The incidence of bacteria among outpatients and inpatient (N=168).

The difference in the distribution of the common organism between outpatients and inpatients was highly significant, P<0.001 (using Chi-square ax2 method).



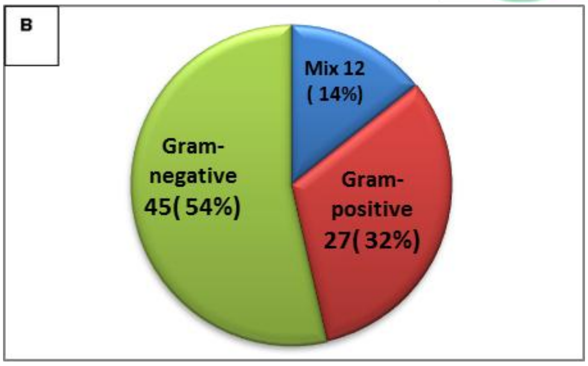


figure 3.The incidence of bacteria among outpatientsA(n=84))and inpatients B (n=84))

TableII. Distribution of the most common organisms amongGram negative and Gram positive isolates

Gram negative isolates			Gram positive isolates		
Bacterial name	**No**	**Percentage**	**Bacterial name**	**No**	**Percentage**
E.coli	36	62	*S.aureus*	76	80.8
Proteus	7	12.1	Beta-haem.Strep.	13	13.9
Klebsiella	8	13.8	*S.faecalis*	5	5.3
P.aeruginosa	7	12.1	-	-	-
Total(152)	58(38.2%)	100	-	94(61.8%)	100

Table III . Age distribution of *S. aureus* and other organisms among outpatients.

Age(year)	*S.aureus*		Other org.		Total	
	No.	(%)	No.	(%)	No	(%)
< 1	3.0	5.3	0	0	3.0	5.3
1-14	18.0	31.6	10.0	37.3	28.0	68.6
15-24	13.0	22.8	5.0	18.5	18.0	41.3
25-44	19.0	33.3	9.0	33.3	28.0	66.6
45-64	4.0	7.0	3.0	11.2	7.0	18.2
Total	57.0	100	27.0	100	84.0	200

There is a difference in the age distribution significant, the distribution of *S. aureus* in comparison with other organisms according to ages was about similar (significant difference).P> 0.001

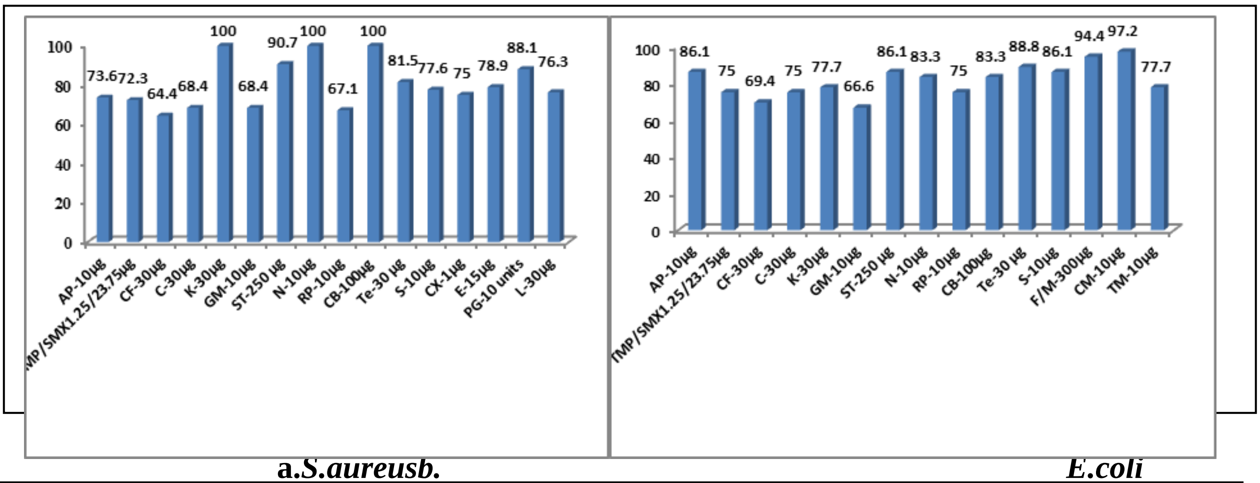
Table IV.Age distribution of *E.coli* and other organisms among hospitalized patients.

Age(year)	E.coli		Other org.		Total	
	No.	(%)	No.	(%)	No	(%)
< 1	3.0	10.7	5.0	8.9	8.0	19.6
1-14	1.0	3.6	1.0	1.9	2.0	5.5
15-24	4.0	14.3	5.0	8.9	9.0	23.2
25-44	7.0	25	21.0	37.4	28.0	62.4
45-64	11.0	39.3	16.0	28.6	27.0	67.9
65-over	2.0	7.1	8.0	14.3	10.0	21.4
Total	28.0	100	56.0	100	84.0	200

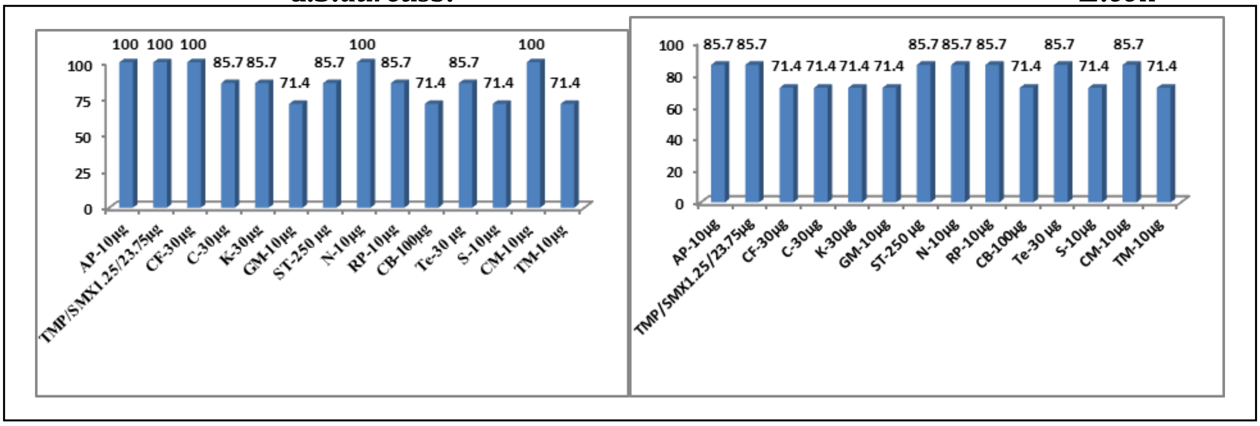
The age distribution of *E.coli* and other organisms was significant, P>0.001.

Al-Kufa University Journal for Biology / VOL.10 / NO.2 / Year: 2018

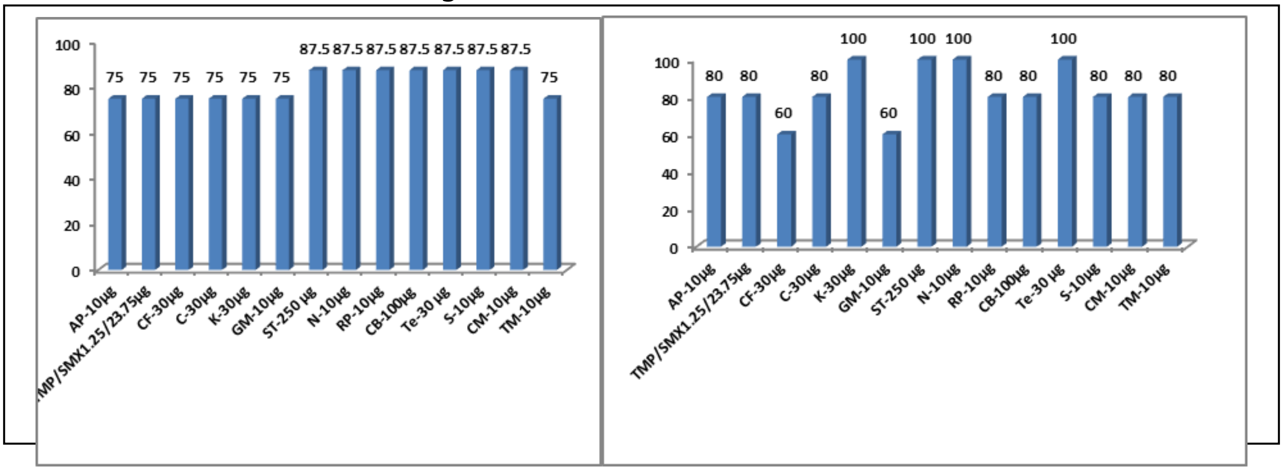
Print ISSN: 2073-8854 & Online ISSN: 2311-6544



a.*S.aureus* b.*E.coli*



c.*P.aeruginosa* d.*Proteus*



e.*K.aerogenes* f .*S. faecalis*

Figure 4. The percentage of antimicrobialresistance of different bacterial isolates from wound infection.

Ampicillin(AP-10µg), Trimethoprim-sulfamethoxazole(TMP/SMX-1.25/23.75µg), Cefotaxime(Cf-30µg), chloramphenicol (C-30µg), Kanamycin(K-30µg), Gentamycin (GM-10µg), Sulfonamides(ST-250 µg),Norfloxacin(N-10µg), Rifampicin(RP-10µg),Carbincillin(CB-100µg), Tetracycline(Te-30µg), Streptomycin(S-10µg),Cloxacillin(cx-1µg), Erythromycin(E-15µg), Penicillin(PG-10 units), Linezolid(L-30µg), Nitrofurantoin(F/M-300µg),Colistin(Co-10µg), Tobramycin(Tm-10µg).

Table V. Antibiotic susceptibility test of β-haemolytic Streptococci

Antibiotics	No. and percentage of isolates
Erythromycin (15)	13(100%)
Ampicillin (10)	11(84.6%)
Penicillin (10 Units)	10(77%)
Chloramphenicol (30)	8(61.5%)
Tetracycline (30)	7(53.9%)
Cefotaxime (30)	6(46.1%)
Streptomycin (10)	6(46.1%)
Cloxacillin(1)	3(23%)

Table VI. The phage-type and groups of the typale strains of *S. aureus*

Phage group	Phage types	No. of strains
I	29	4
	29/52	1
	52	1
	80	1
	79	2
II	3C	6
	3C/55	3
	3C/55/71	1
	3A/3C/55/71	2
	71	1
III	42E/54	1
Unclassified	29/3A	1
	52/3A	1
	29/3C	1
	29/3A/3C	1
	29/3A/3C/71	1

TableVII. The correlation between *Staph.aureus* phage 3C and different antibacterial agents used.

Staph.aureus **code number**	**Age(year)**	**Sex**	**Position of the wound**	**Antibiotic diagram**
15	30	male	Leg	RF, TMP/SMX, GM, C, CXP, L, S
91	17	male	Hand	RF, CF,E , TMP/SMX, GM, S, N, L
39	28	male	Axilla	RF, CF, TMP/SMX, AM, L, CX, S
45	17	female	Hand	RF, CF, TMP/SMX, GM, CX, TE, L
67	1.5	male	Head	RF, CF, TMP/SMX, E, TE, L, GM, C
70	5	male	Face	RF, CF, TE, S, CX, GM, C, N,P

References:

1- Cooper, R.; Kingsley, A.and White, R. (2003).Wound Infection and Microbiology. Medical Communications (UK) Ltd. for Johnson & Johnson Medical.

2- Sule, A.; Thanni, L.; Sule-Odu, O. and Olusanya, O. (2002).Bacterial pathogens associated with infected wounds in Ogun state university teaching hospital, Sagamu, Nigeria. Afr J ClinExpMicrobiol;3:13-16.

3- Mulugeta,K.A. and Bayeh, A.B.(2011).Bacteriology and antibiogram of pathogens from wound infections at Dessie Laboratory, North East Ethiopia. *TJHR.,13;4.*

4- Lipsky, B.A.; Berendt, A.R.; Cornia, P.B.; Pile,J.C.; Peters,E.J.G.; Armstrong,D.G.; H.G., and Senneville, E. (2012). Infectious diseases society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infectionsa.Clinical Infect. Dis. 54 (12):132 173.

5- Verma,P.(2012). A study on isolation of different type of bacteria from pus. Int. J. of Pharm. & Life Sci. (IJPLS). 3,(11):2107-2110.

6- Parajuli, P.;Basnyat, S.R.; Shrestha, R.; Shah, P.K.and Gurung, P. (2014). Identification and Antibiotic Susceptibility Pattern of Aerobic Bacterial Wound Isolates In Scheer Memorial Hospital. JSM Microbiology 2(2): 1011.

7- Sulaiman, A. A.andZayed, M.E.(2014). Antibacterial susceptibility of bacteria isolated from burns and wounds of cancer patients. J S C S.18, 3–11.

8- Amatya, J.; Rijal, M. and Baidya, R.(2015). Bacteriological Study of the Postoperative Wound Samples and Antibiotic Susceptibility Pattern of the Isolates in B&B Hospital. JSM Microbiology 3(1): 1019.

9- Akhi, M. T.; Reza, G.; Samad, B.; Mohammad, A. and other(2015).Antibiotic Susceptibility Pattern of Aerobic and Anaerobic Bacteria Isolated From Surgical Site Infection of Hospitalized Patients. Jundishapur J Microbiol.; 8(7): e20309.

10- Verma,P.(2012).Antibiotic Sensitivity Treatment for Gram Positive Bacteria Isolated from Pus Sample. Bull. Environ. Pharmacol. Life Sci.1(10): 03 – 06.

11- Parajuli, P.;Basnyat, S.R.; Shrestha, R. Shah, P.K.andGurung, P.(2014). Identification and Antibiotic Susceptibility Pattern of Aerobic Bacterial Wound Isolates In Scheer Memorial Hospital. JSM Microbiology 2(2): 1011.

12- Rajalakshmi,V. and Amsaveni, V. (2012). Antibiotic Susceptibility of Bacterial Pathogens Isolated from Diabetic Patients, I J Microbiol. Research,3(1): 30-32.

13- Koneman, W.K.; Allen, S.D.;Janda, W.M.;Schreckenberger, P.C.;Propcop, G.W.;Woodsand, G.L. and Winn, W.C.(2005). Jr. Philadelphia Color Atlas and Textbook of Diagnostic Microbiology, 6th ed. Lippincott- Raven Publisher, 624-662.

14- Cheesbrough, M.(2006): District laboratory practice in tropical countries volume II: microbiology. Cambridge (UK): Cambridge University Press; 2006:1–479.
15- Elantamilan, D.A.; Bakorlin, K.; Wihiwot, V. L.; Jyotismita, R. and Debahuti S.(2016).Characterization of beta- hemolytic streptococci from clinical isolates in a tertiary care centre in North-East India. IntJ.HealthSci Res. 2016; 6(1):165-175.
16- Denise,I.;Spencer,J.; Melissa,M. and Patricia, A.C.(2009). Characterization of beta-hemolytic streptococci isolated from southern sea otters (Enhydralutrisnereis) stranded along the California coast. Veterinary Microbiology 136 (2009) 378–381.
17- Clinical and Laboratory Standards Institute (CLSI). (2012).Performance Standards for Antimicrobial Susceptibility Testing; 22^{ed}. Informational Supplement.32 (3).
18- Opalekunde,A.B.; Adesiji,Y.S.; Bukoye, Y.D. and Ajao,A.T.(2014). Prevalence And Drug Sensitivity Pattern Of Isolates From Wound Infection In Some Selected Hospitals In Kwara State, Nigeria. Report and Opinion 2014;6(8)
19- Mohammed, A.; Adeshina, G. O. and Ibrahim, Y. K. E.(2013). Retrospective incidence of wound infections and antibiotic sensitivity pattern: A study conducted at the Aminu Kano Teaching Hospital, Kano, Nigeria.IJMM S. 5(2), pp. 60-66.
20- Rao,R.; Sumathi, S. andAnuradha,K.(2013). Bacteriology of postoperative wound infections. IntJpharm biomed res,4(2), 72- 76.
21- Shriyan, A.; Sheetal, R. and Nayak, N.(2010). Aerobic micro-organisms in post-operative wound infections and their antimicrobial susceptibility patterns. Jcdr., 4, 3392-3396.
22- Mohammad,S.R.; Anil, C. and Abirodh R.(2013). Antimicrobial susceptibility patterns of the bacterial isolates in post-operative wound infections in a tertiary care hospital, Kathmandu, Nepal. Open journal of medical microbiology 3, 159-163.
23- Devi, U.; Prasanta, K.B.andJagadish M.(2011).The prevalence and antimicrobial susceptibility patterns of beta-hemolytic streptococci colonizing the throats of schoolchildren in Assam, India. JI DC.; 5(11):804-808.
24- Nazeer,H.A.;Shaik, K. M.andKolasani,B.P.(2014). Aerobic bacteriology of wound infections with special reference to MRSA.Journal of Clinical & Experimental Research.2:1.
25- Ahmed,J.; Ali, A.andHussain, M.(2013). Antimicrobial susceptibility pattern of bacteria isolated from burn wounds in a tertiary care hospital in Pakistan. *African journal of microbiology research*, 7(28), 3627-3631.
26- Bayram,Y.; Parlak, M.and Aypak, C.(2013).Three year review of bacteriological profile and antibiogram of burn wound isolates in Van, Turkey. International journal of medical sciences, 10(1), 19-23.
27- Magnet,M. H.; Arongozeb, M.d. ; Khan, G. M. and Zakaria, A.(2013). Isolation and Identification of Different Bacteria from Different Types of Burn Wound Infections and Study Their Antimicrobial Sensitivity Pattern. IJRANSS. 1(3), 125-132.
28- Nayak, N.; Lenka, R.K. and Padhy R. N.(2014). Surveillance of multidrug resistant suppurative infection causing bacteria in hospitalized patients in an Indian tertiary care hospital.Journal of Acute Disease 148-156.
29- Ahmed,M.I.(2012). Prevalence of Nosocomial Wound Infection AmongPostoperative Patients and Antibiotics Patterns at Teaching Hospital in Sudan. North Am J Med Sci.4(1),29-34.
30- Raza, M. S.;Chander, A. and Ranabht, A.(2013). Antimicrobial susceptibility patterns of the bacterial isolates in post-operative wound infections in a tertiary care hospital, Kathmandu, Nepal. Open journal of medical microbiology 3, 159-163.
31- Bowler, C., Chigbu, O. C. and Giacometti, H. (2001). Emergence of Antimicrobial Resistance Bacteria. Journal of Antimicrobial and Chemotherapy 23:12 – 23.

32- Ratemo,N.(2014). Antimicrobial Susceptibility Pattern of Bacterial isolates from Pus samples at Kenyatta NationalHospital, Kenya.MSc. thesis.Univ. of Nairobi.

33- Vieira, M. A.; M. Ruth; Vicente, P.andJuliana,L.(2014). Methicillin-resistant Staphylococcus aureus nasal carriage in neonates and children attending a pediatric outpatient clinics in Brazil. braz j infect dis.18(1):42–47.

34- Mawalla, B.;Mshana, S.E.;Chalya, P.L.;Imirzalioglu, C.andMahalu, W.(2011). Predictors of surgical site infections among patients undergoing major surgery at Bugando Medical Centre in Northwestern Tanzania. BMC Surgery.11:21.

35- Anderson, D.J.; Sexton, D.J.; Kanafani, Z.A.; Auten, G.and Kaye, K.S.(2007).Severe surgical site infection in community hospitals: epidemiology, key procedures, and the changing prevalence of methicillin-resistant *Staphylococcus aureus*. Infect Control Hosp Epidemiol.28:1047-53.

36- Godebo, G.; Kibru, G. and Tassew, Himanot.(2013). Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. Annals of Clinical Microbiology and Antimicrobials.12(1)17.

37- Muluye, D.;Wondimaenh,Y.andFerede,G.(2014). Bacterial isolates and their antibiotic susceptibility patterns among patients with pus and/ or wound discharge at Gondar university hospital. *BMC research notes,* 7, 1-5.

38- Dessalegn, L.; Shimeis, T.and Endale T.(2014). Aerobic bacterial isolates from post-surgical wound and their antimicrobial susceptibility pattern: a hospital based cross-sectional study. E3 journal of medical research.3(2) 18-23.

39- Moyo, S.; Aboud, S.; Kasubi, M.andMaselle, S. Y.(2010). Bacteria isolated from bloodstream infections at a tertiary hospital in Dares Salaam, Tanzania – antimicrobial resistance of isolates. S Afr Med J;100:835-8.

40- Dhanda, V .; Chaudhary, P.and Toor,D.(2013). Antimicrobial susceptibility pattern of betahaemolytic group A, C and G streptococci isolated from North India. Journal of Medical Microbiology . 62, 386–393.

41- Acharya, J .;Mishra,S.K.; Kattel, H. P. and Rijal , B.(2008). Bacteriology of wound infections among patients attending tribhuvan university teaching hospital ,Kathmandu,Nepal. JNAMLS .9(1) *76-80.*

42- Kali,A.; Stephen, S.; Umadevi, S.and Kumar, S.(2013). Bacteriophage types of methicillin-resistant *Staphylococcus aureus* in a tertiary care hospital. AMJ 2013, 6, 10, 496-503.

43- Mehndiratta, P.L.; Gur, R.; Saini, S.andBhalla, P. (2010).*Staphylococcusaureus* phage types and their correlation to antibiotic resistance. Indian J Pathol Microbiol.53:738-41.

44- Murchan, S.; Carter, M. and Aucken, H.M.(2000). Strain identities of phage non-typable MRSA in the UK. J Hosp Infect.46:157-8.

45- Manyahi, J.(2012). Bacteriological Spectrum of Post operative wound Infections and their Antibiogram in a tertiary hospital, Dar es Salaam, Tanzania.M.Sc thesis. Muhimbili University