# Study of Pathogenic Bacterial Isolates from Patients with Skin Infections and their susceptibility to Antibiotics in Some Hospitals in Taiz City-Yemen

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**Abstract:**

**Background:** Skin and soft tissue infections (SSTIs) are commonly encountered in clinical practice, the spectrum of the causative bacterial agent changes constantly and so does their antibiotics.
**Aim:** This study was carried out to investigate the incidence of different aerobic bacteria isolates.
**Materials and methods:** Samples were collected from patients with skin infections at a local Hospital in Taiz City, Yemen. A cross-sectional study was conducted during the period between January to December 2014. A pus specimens were collected aseptically from 130 cases of skin and soft tissue infections in the sterile condition and cultured on blood agar, MacConkey agar and mannitol-salt agar media. After growing and staining of bacteria by gram stain, bacteria were cultured in differentiated media, strains that were isolated, undergo antibiotic susceptibility test by Kirby-Bauer disc diffusion method.
**Results:** From a total of 130 swabs collected, 89 (68. 5%) were with bacterial growth : gram positive bacteria form [57 (64%)], while gram negative bacteria were [32 (36%)], common aerobic bacterial pathogens were: *S. aureus*[30 (33.7%)], *S. epidermidis* [18 (20.2%)], *P. aeruginosa* [14 (15.9%)], *S. pyogenes* [9 (10.1%)], *E. coli* [8 (8.9%)], *K. Pneumoniae* [6 (6.7%)], *P. mirabilis* [4 (4.5%)]. These results showed that in vitro antibiotic susceptibility tests among gram positive cocci susceptibility was highest to Vancomycin, Fusidic acid (except S. pyogenes) and Ciprofloxacin. Among gram negative bacilli Gentamycin, Chloramphenicol and Ciprofloxacin susceptibility was high, however, all *Escherichia coli* isolates were resistant to Penicillin, Ampicillin, and Tetracycline. Additionally, all *Proteus mirabilis* isolates were resistant to Erythromycin and Penicillin.
**Conclusion:** This study indicated that the multi drug resistance (MDR) of bacterial organisms were alarming for increase in skin infections .
**Keyword:** Pathogenic bacteria, Skin and soft tissue infections, Antibiotic resistance, Taiz City, Yemen.

## Introduction

The human skin and soft tissue infections caused by microbial pathogens during or after trauma, burn injuries, and surgical procedures result in the production of pus, a white to yellow liquid contained dead WBCs, cellular debris, and necrotic tissues**[1-3].** Human skin has innate properties that are important in preventing infection and promoting healing in wounds. The structure and function of the skin are not uniform, and specific adaptations are found at different anatomical sites. Human skin is a multifunctional organ that provides sensation, thermoregulation, biochemical, metabolic, immune functions, and physical protection **[4].** Both aerobic and anaerobic bacteria have been implicated in wound contaminations, which commonly occur under hospital condition and result in significant morbidity, prolonged hospitalization, and huge economic burden **[5].** The most commonly isolated bacterial pathogens are Staphylococcus aureus, coagulase-negative Staphylococci (CNS), Enterococcus sp. And Escherichia coli (E. coli);however, the pathogens isolated depend on the surgical procedure **[6].**

Antibiotics are one of the mainstays of modern medical care and play a major role in the prophylaxis and treatment of infectious diseases **[7],** but the widespread uses of antibiotics, together with the length of time during which they have been available have led to major problems of resistant organisms contributing to morbidity and mortality **[8].** The current spread of multi-drug resistant bacterial pathogens has added a new dimension to the problem of skin infections **[9].** This is especially more terrible in resource-poor countries where the sale of antibiotics is poorly controlled **[10].**

## Aims of the study

The current study was conducted to find the pathogenic bacterial agents and determine their antibiotic susceptibility pattern in cases of the human skin and soft tissue infections.

## Materials and Methods

This study was conducted in Taiz city, during the period between January to December 2014, 130 pus specimens were collected by sterile swabs from patients in different Hospitals in Taiz City -Yemen. The sources of specimens were pus/swab from wound (64), boils (45) and abscesses (21), infected skin was cleaned with normal saline and a swab of wound secretion/pus, purulent exudates, or wound discharge was aseptically obtained using a sterile cotton swab from each study participant. The specimen was collected on a moistened cotton swab without contaminating with skin commences and the swab was immersed in a container of transport medium. Soon after collection, each sample was transported to the laboratory. The specimens collected from males and females with varied ages and none had been treated with antibiotics during the previous one week time.

**Culturing of Urine Specimens:** Firstly, the wound swab was used to make Gram stain smears, it was inoculated into blood agar, MacConkey agar and mannitol-salt agar. All the plates were incubated aerobically and initially examined for growth

after 24 hrs and the ones without growth were further incubated for up to 48 hrs**[11].**

**Identification of the Isolates:** Identification of Gram positive bacteria was done using Gram stain, hemolytic activity on sheep blood agar plates, catalase reaction and coagulase test for Gram-positive bacteria. Gram-negative bacteria were identified based on colony morphology on blood agar and MacConkey agar, followed by biochemical reactions, namely oxides, IMVC test (I= Indol, MR= Methyl red VP= Voges – Proskauer, C= Citrate utilization), motility and urease tests**[12,13,14].**

**Detection of Hemolysin production (β –hemolysis) by bacterial skin pus isolates:**

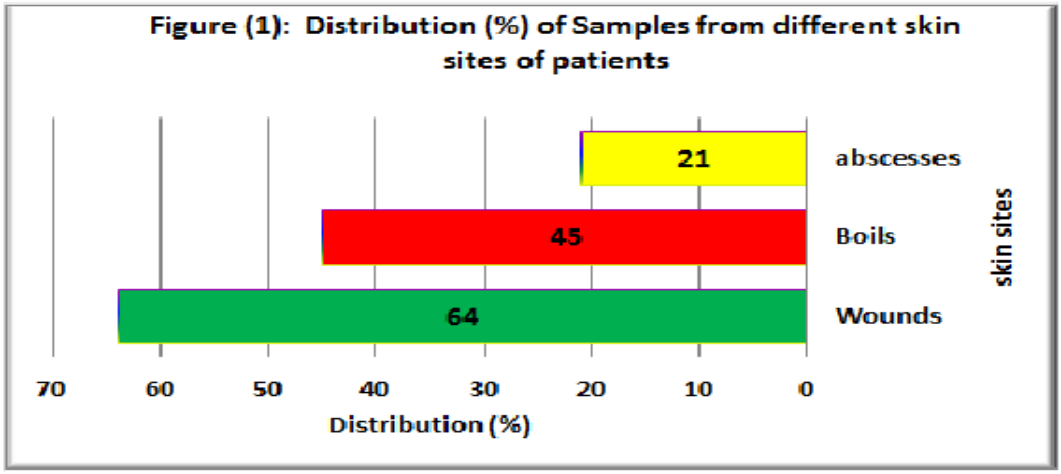
All isolates were tested for blood hemolysis using blood agar plates containing 5% (v/v) human blood and incubated aerobically at 37 $^{O}$C for 24 hours **[15].**

**Antibiotic Susceptibility Test:**

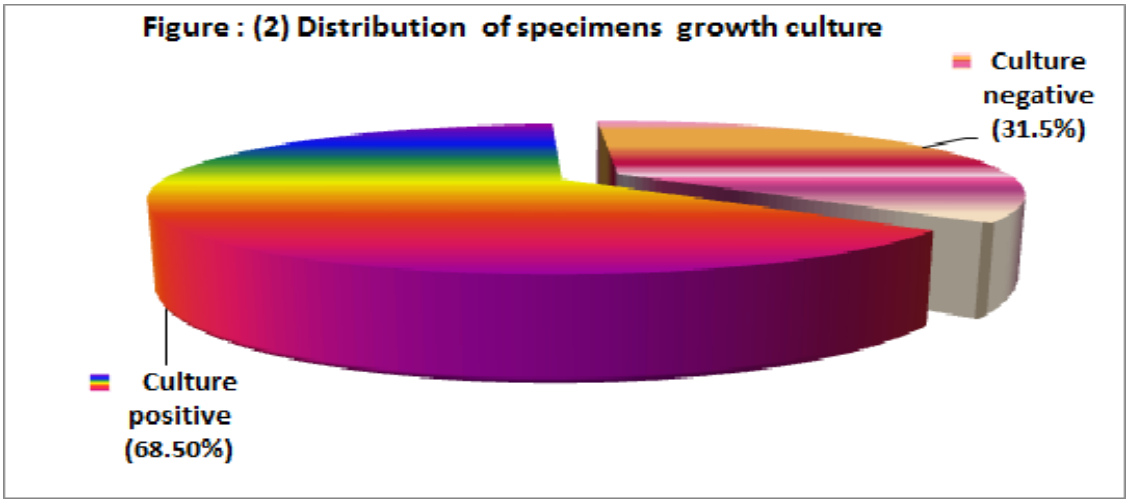
Following identification of the bacterial isolates, antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar as described by the National Committee for Clinical Laboratory Standards (presently called Clinical and Laboratory Standards Institute(CLSI) **[16]**. The isolates were tested against commonly prescribed antibiotics: 10 IU Penicillin (PG), 30 µg Ampicillin (AMP), 25µg Amoxacillin (AMX), 30µg Fusidic acid (FD), 30µg Cefotaxime (CTX), 5µg Erythromicin (ERY), 30µg Chloramphenicol (CHL), 10µg Gentamicin (GEN) and 30µg Tetracycline (TET), 5µg Ciprofloxacin (CIP), 30µgVancomycin (VA), sensitivity was read after incubation for 24 hrs. at 35ºC.The bacteria isolates were regarded as sensitive or resistant according to CLSI criteria**[16].** Fusidic acid and Vancomycin antibiotics were used for Gram positive isolates only, Novobiocin disks were used to distinguish *Staphylococcus epidermidis*, which is sensitive to novobiocin in culture, from other coagulase-negative *Staphylococci* (CONS: *Staphylococcus saprophyticus*) and Bacitracin disks were used to distinguish *S. pyogenes* (β-hemolytic streptococci), which is sensitive to Bacitracin in culture, from *S. agalactae* (β-hemolytic streptococci) .

**Results**

The source of swabs are shown in **Figure .1.**

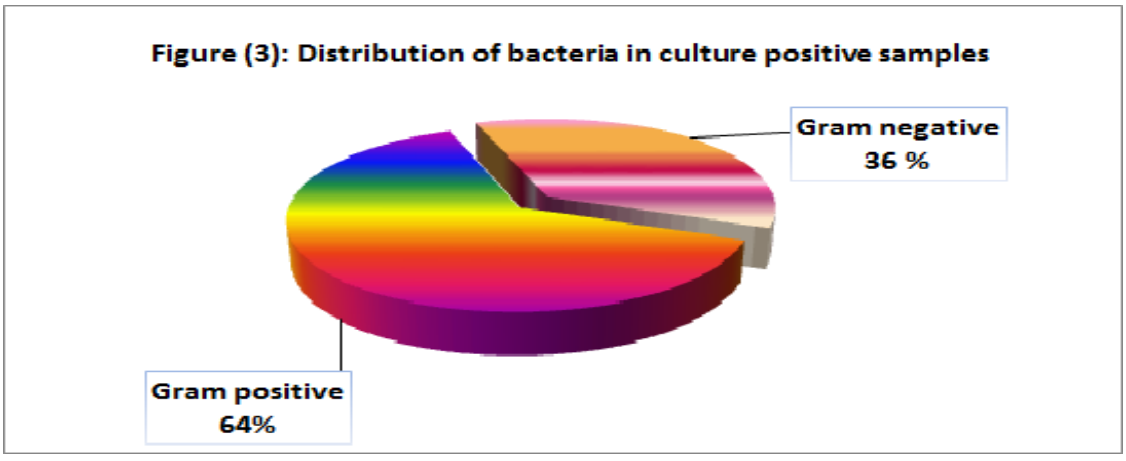



Among these infections, about 85 (65.4%) were males and 45 (34.6%) were females with different ages, 89/130 (68.5%) specimens showed bacterial culture positive after 24–48 h of incubation. Whereas 41/130 specimens (31.5%) were



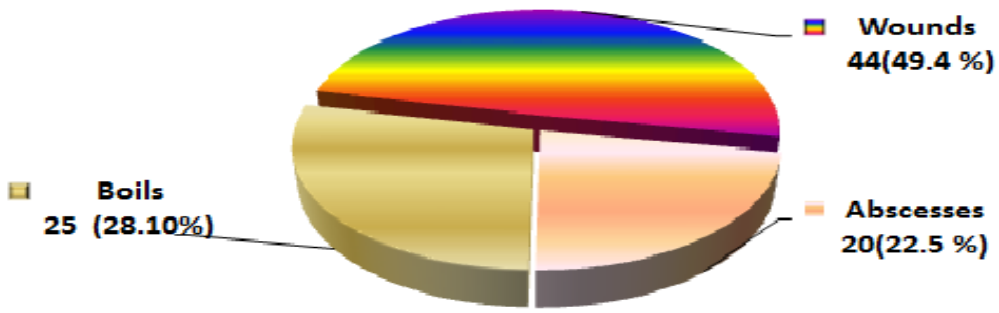

bacterial culture negative, **Figure .2.**

Among the positive isolates, 57/89 (64%) were Gram positive and 32/89 (36%) were Gram negative, **Figure .3.**



The most prevalent of clinical were wound as[ 44/89, (49.4 %)] followed by Boils [25/89 (28.1 %)] and the least was abscesses [20/89 (22.5 %)],which showing in **Figure .4.**

**Figure (4): Distribution of cases of Skin infections**




Based on Gram staining, morphological features, cultural characteristics and biochemical characterization, the bacterial isolates were assigned to seven bacterial species. *S. aureus* was the most frequent pathogen as revealed by (33.7%) followed by *S. epidermidis* (20.2%), *P. aerogenosa* (15.9%), *S. pyogenes* (GroupA*Streptococci*)(10.1%), *E. coli* (8.9%), *K. pneumoniae* (6.7%) and *P.mirabilis* (4.5%). Gram-positive bacteria were the predominant pathogen among both sexes (53.9%), followed by negative bacteria (all isolates found in male patients were also found in female patients except for *P. mirabilis*, which was only found in a female patient. From these, 89 positive isolates 51/89 (57.3%) were males and 38/89 (42.7 %) were females with skin infections and our findings showed there were no relationship between gender and the skin infections through all clinical isolates, **Tables (1, 2) and Figures (5 , 6)**

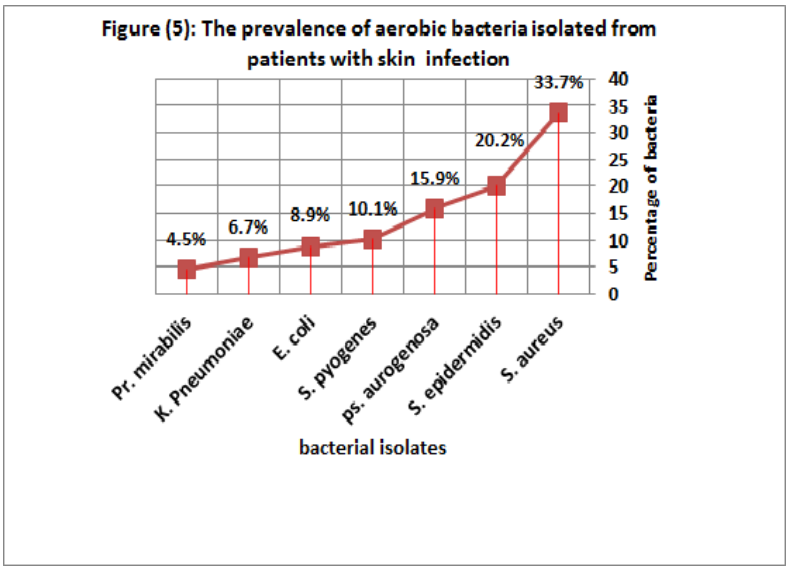
Table (1 ) Some of differential Biochemical test for gram-positive cocci isolates from skin infection

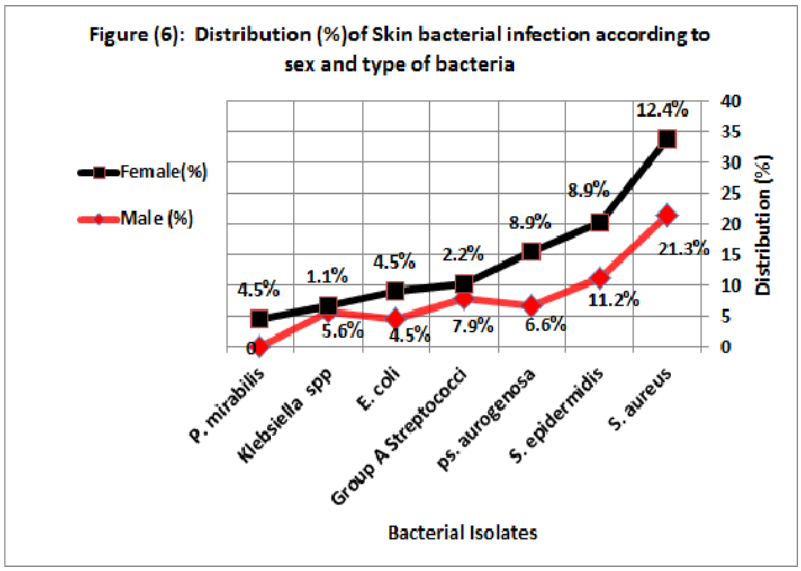
| Tests /Bacteria | Result of Tests | | |
|---|---|---|---|
| | *S. aureus* | *S. epidermidis* | *S. pyogene* |
| Gram Stain | +ve | +ve | +ve |
| Catalase reaction | +ve | +ve | -ve |
| Coagulase reaction n | +ve | -ve | ND |
| Oxidase reaction | -ve | -ve | -ve |
| Typical of hemolysis | Beta | None | Beta |
| Growth on Mannitol | +ve | -ve | -ve |

Table (2 ) Some of differential Biochemical test for gram-negative cocci isolates from skin infection

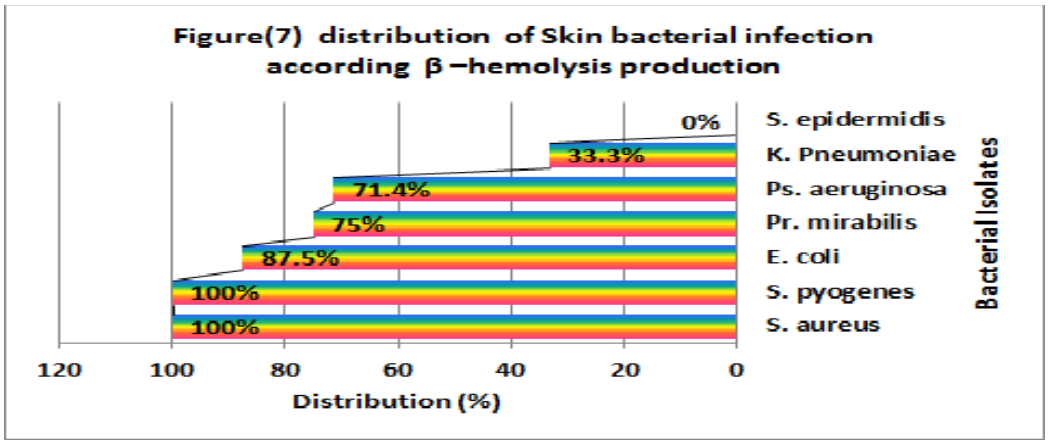
| Tests /Bacteria | Result of Tests | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Gram Stain | Catalase | Oxidase | Urease | Motility | I | MR | VP | C |
| *E.coli* | -ve | +ve | -ve | -ve | +ve | +ve | +ve | -ve | -ve |
| *K. Pneumoniae* | -ve | +ve | -ve | +ve | -ve | -ve | -ve | +ve | +ve |
| *Ps.aurogenosa* | -ve | +ve | +ve | +ve | +ve | -ve | +ve | -ve | +ve |
| *Pr mirabilis* | -ve | +ve | -ve | +ve | +ve | -ve | +ve | D | D |

+= positive, - = negative, D= different

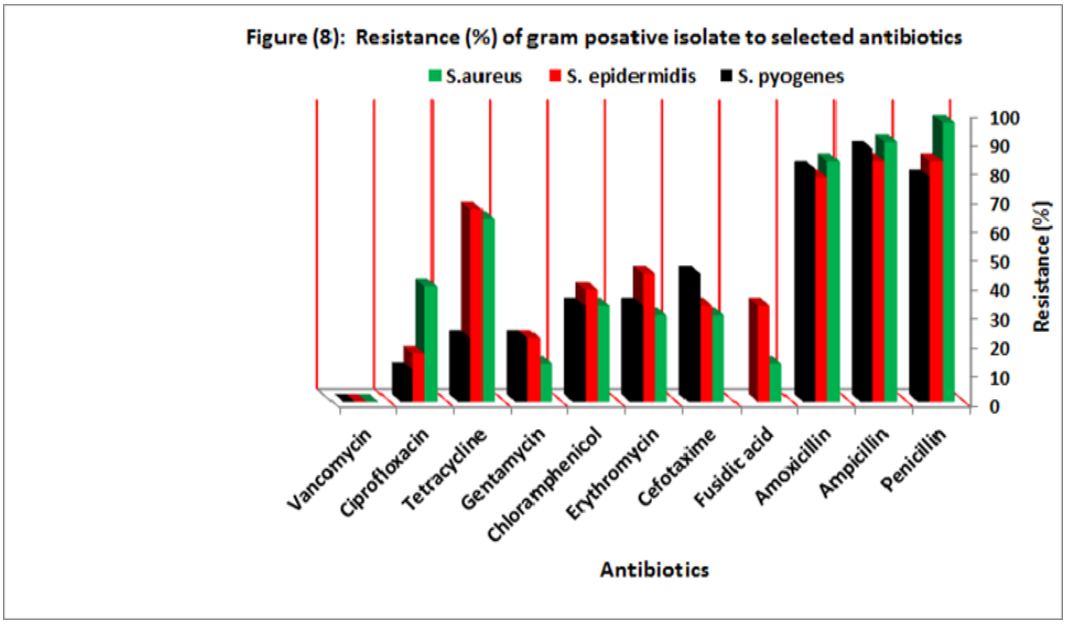



Also, the present study shows that the appearance of clear zones of hβ – hemolysis after the end of the incubation period around colonies on blood agar plate with different diameters were appeared as (100%) by *S. aureus* and by *S. pyogenes*, followed by *E. coli (*87.5%), *P.mirabilis* (75%), *P. aeruginosa* (71.4%) and *K. Pneumoniae* (33.3%), while *S. epidermidis* isolated did not produce β – hemolysis, indicated by no color changes around the bacterial colonies on blood agar, **Figure .7.**

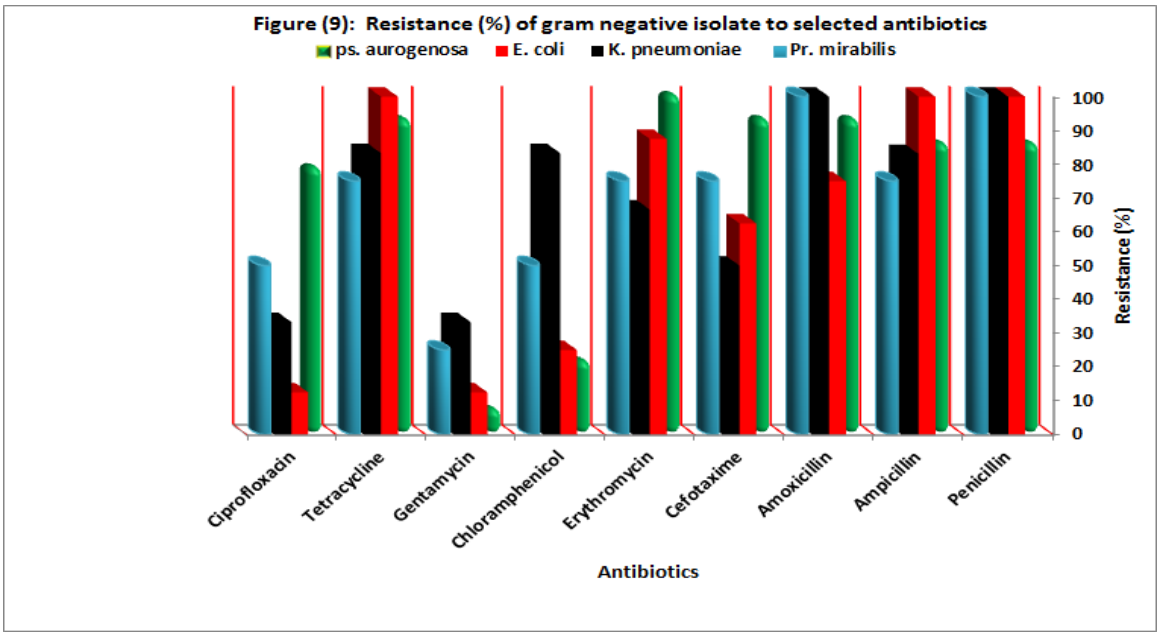



Finally, antibiotics susceptibility test for all skin infections showed that the most isolated pathogenic bacteria were with multiple antibiotic resistance activities to the tested antibiotics. *S. aureus* isolates showed maximum resistance against penicillin (96.7%), followed by Ampicillin (90%), Amoxicillin (83.3%) and Tetracycline (63.3%), but all isolates were sensitive (100%) to Vancomycin, while other antibiotic sensitivity were (86.7%) to Fusidic acid and Gentamycin. Cefotaxime and Erythromycin susceptibility was (70%), followed by Chloramphenicol and Ciprofloxacin as (66.7, 60 %). *S. epidermidis* isolates show a high resistance to Penicillin and Ampicillin (83.3%), while to Amoxicillin and Tetracycline demonstrated resistance rate of (77.8) and (66.7 %) , respectively. Additionally, the susceptibility to Ciprofloxacin was (83.3%) , followed by Gentamycin as (77.8%). *S. pyogenes* were most resistant to Ampicillin (88.9%), Amoxicillin (77.7%) and Penicillin (77.8%), whereas showed different susceptibility to the other tested antibiotics for Gram positive bacteria except Fusidic acid and Vancomycin which were not tested, **Figure.8.**



All *Ps.aeruginosa* isolates were 100% resistant to Erythromycin, followed by Amoxicillin, Cefotaxime. But, Tetracycline resistant was (92.9%), Penicillin and Ampicillin was (85.7%) and Ciprofloxacin was (78.6%). While isolates were sensitive to Gentamycin and Chloramphenicol in [(92.9),(78.6%)], respectively. *E. coli* isolates were 100% resistant to Penicillin, Ampicillin and Tetracycline, followed by Erythromycin, Amoxicillin, Cefotaxime as [(87.5%), (75%), (62.5%), respectively. But, *E. coli* isolates showed (87.5%) sensitivity to Gentamycin and Ciprofloxacin.

All *K. pneumonia* isolates were (100%) resistance to Penicillin and Amoxicillin, followed by (83.3%) for Ampicillin, Chloramphenicol and Tetracycline. They were with (66.6%), (50%) resistance rate to Erythromycin and Cefotaxime, respectively, while with sensitivity rate of 66.3% to Gentamycin, Ciprofloxacin. *Pr. mirabilis* isolates showed (100%) resistance to Penicillin and Amoxicillin, followed by (75%) resistance to Ampicillin, Cefotaxime, Erythromycin, and Tetracycline. A resistance rate of 50% to Ciprofloxacin and Chloramphenicol, while 75% of *Pr. mirabilis* isolates were sensitive to Gentamycin , **Figure .9.**




## Discussion

In the present study the culture positively rate was [89/130 (68.5%) ], this result was similar to the reported studies from Saudi Arabia (65.74%) [17], and from India (97.01%) [18]. The prevalence may be the result of different infection control practices and general hygiene in the investigated hospitals. Also, bacterial growth was not seen in [41/130 (31.3%)] patients, which could attribute to the normal healing process of the wound under the influence of the host immune responses, antimicrobial activity or appropriate use of antiseptics for cleaning the wounds. However, negative culture may be due to anaerobic bacteria or fungus infection, which missed due to the use of culture media that only support the

aerobic bacteria [19, 20]. In this study, gram-positive pathogens isolated from skin infections in (64%) and Gram negative bacteria in (36%), this is in consistent with the results reported by others [21-23]. But not agreed with Olowe*et al.* [24], who reported that the rate of bacterial isolate among clinical skin infections was (85.7%), out of that (61.4%) of the isolates were gram negative bacteria and (38.6%) of the isolated were gram positive cocci. The differences in the prevalence of Gram positive and negative in wound infections may be attributed to different factors such as the patient population, microbial community in patients' bodies, procedures and number of specimens, hospitalization stay period, and the distribution of specimen collected during the study period. Importantly it is known that Gram positive microorganisms, usually are isolated in the early stages of hospitalization, while in prolonged hospitalization while Gram negative microorganisms predominate, as the results of nosocomial infection [25]. In addition our findings indicated that the most widely recognized pathogens isolated were gram-positive cocci, such as *S.aureus* (33.7%), followed by *S. epidermidis* (CONS) as (20.2%) and *S. pyogenes*as (15.7%). While the most common pathogens isolated of gram-negative rods was *Ps.aeruginosa* (15.7%), followed by *E. coli* (9%), *K. Pneumoniae* (6.7%) and *P.mirabilis* (4.5%). These results were agreed to the report of CDC, which indicated that *S.aureus* is the most prevalent bacterium associated with skin infections. Infection with *S. aureu*s most likely associated with endogenous source as it is a member of the skin and nasal normal flora and also exogenous source with contamination of environment, surgical instruments or from the hands of health workers [26,27,28].In addition to being normal flora on human skin, the CONS are important nosocomial pathogens, often multidrug-resistant and have become disseminated worldwide [29]. *P. aeruginosa*is as an opportunistic nosocomial pathogen, which causes a wide range of infections and leads to substantial morbidity in immunocompromised patients and due to its high drug resistance to many antibiotics, the mortality rate is substantial [30, 31]. Moreover, the aerobic gram-negative bacteria (mainly *Enterobacteriaceae* and sometimes *P. aeruginosa* or other gram-negative species) are usually isolated in conjunction with gram-positive cocci in patients with chronic or previously treated infections [26], which is consistent with our findings. Basically, it seems that *S. aureus* and *P. aeruginosa* produce different virulence factors and display innate resistance against different drugs and are known to be major causes of wound infection in hospitalized patients.

This study shows that (61) bacterial isolates (68.5%) produced β-hemolysin, many types of bacteria have able to produce β- hemolysin when cultured on blood agar and produces zones of hemolysis that are only slightly larger than the colonies themselves [32]. Hemolysin production was indicater of the bacterial virulence and thus 68.5% of the causative bacteria for wound infections were with high virulence. This important virulence factor which is cytotoxic due to the formation of trans membranous pores in the host cell membrane [33]. Most strains of *S. aureus* will exhibit β-hemolysis when grown on

blood agar which can be a distinguishing characteristic. *S. aureus*is differentiated from other staphylococcal species on the basis of coagulase reaction (coagulase positive) [34] and furthermore produce by many strains of *Proteus* and *P. aeruginosa* understanding [35]. Regarding to antimicrobial susceptibility, Multidrug-resistant bacterial infection becomes a real threat in developing countries, including Yemen (especially hospitals of the Taiz City). Antimicrobial resistance pattern of gram positive cocci isolated from skin infection In the present study, gram positive bacteria demonstrated elevated amounts of resistance [(71.9–89.5%)] to amoxicillin, ampicillin and penicillin, these finding is similar to the studies carried out in India [36], which show resistance rate of were 75–100% to the above antibiotics. Additionally, *S. aureus* resistance rate to amoxicillin, ampicillin and penicillin was (83.3–96.7%), which is too high. These outcomes are in concurrence with the reports from Ethiopia and different countries [37,38,39]. The high occurrence of multiple antibiotic resistance may be an appropriate utilization of antimicrobials, absence of laboratory diagnostic tests, unavailability of guideline for the selection of antibiotics. Outbreaks of gram positive cocci resistant to beta-lactam antibiotics have been frequently associated with devastating nosocomial infections [39,40]. Bacterial resistance to beta-lactam antibiotics are primarily due to the production of beta-lactam ring of the antibiotics rendering them inactive [41]. Inappropriate practices like misuse and abuse of antibiotics and unskilled practitioners can also lead to the emergence of resistance in bacteria. Expired antibiotics, self-medication, counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates [40]. Also gram positive isolates showed resistance to Tetracycline (57.9%) and all isolates were (100%) sensitive to vancomycin, followed by Fusidic acid (except S. pyogenes), Gentamycin (82.5%), Ciprofloxacin (71.9%), Erythromycin, Chloramphenicol (65%). This is in correlation with the study of Thind et al, where *S. aureus* showed (100%) sensitivity to vancomycin [42]. Majority of *S. pyogenes* were resistant to erythromycin (50%) in the present study. Judge et al, observed a similar pattern of resistance of *S. pyogenes* to erythromycin48% [43]. Fusidic acid was relatively effective antibiotics against Gram positive organisms associated with skin infection which is in agreement with previous studies conducted in both Europe and North America [44, 45, 46]. While this study shows less resistance (27.8%) to Gentamicin similar to study performed elsewhere [47,48].

*P. aeruginosa, E. coli, K. pneumonia* and *P.mirabilis* demonstrated high level of resistance to most of the antibiotics tested. Antibiotics results from the present study show that the isolates were showed high levels of resistance (50–93.8%) to Ciprofloxacin (except K. Pneumonia, and E. coli), Cefotaxime, Erythromycin, Ampicillin, Tetracycline, Amoxicillin and Penicillin. While show different sensitivity to Gentamycin and Chloramphenicol (except *Klebsiella spp* (16.7%), P. mirabilis (50%)) and Ciprofloxacin [except *P.aeruginosa (*21.4%) ,*P. mirabilis*(50%)]. Majority of gram negative bacteria showed very high resistance to Penicillin, Amoxicillin, Tetracycline, Ampicillin, Erythromycin, Cefotaxime,

which is in agreement with other studies worldwide [49, 50]. The high rate of bacterial resistance against ampicillin, and amoxicillin is likely due to frequent use of these antibiotics both within hospital and outside, incomplete course , and/or low dose.. This high resistance of organisms to the most commonly used antibiotics (*β*-lactam antibiotics) was reported from many studies [51, 52]. The *Enterobacteriaceae* family was highly resistant to the majority of antibiotics tested, which is partially consistent with the findings of the study of Banashankari *e tal.* in 2012 [53]. Moreover, Proteus spp. were resistant to all beta-lactamase antibiotics and gentamicin. *Proteus spp*. are known to produce a unique β-lactamase (cefuroximase) that has a high activity against antibiotics, primarily cefotaxime [55], a third-generation cephalosporin.

*E. coli* isolates were resistant to the majority of antibiotics tested, with the exception of gentamicin. In this way,, in our study, gentamicin, Chloramphenicol and Ciprofloxacin were the most effective antibiotics against almost all bacteria from the *Enterobacteriaceae* family, which is partially consistent with the results of previous studies [54,56]. It is important to consider that some gram-negative bacteria from the Enterobacteriaceae family have the ability to produce highly effective ß-lactamase enzymes, making them resistant to all ß-lactam antibiotics, except cephamycins (cefoxitin, cefotetan) and carbapenems [57]. In 2011, Sivanmaliappan and Sevanan reported that (100%) of *P. aeruginosa* isolates were resistant to ampicillin, (83.3%) resistant to tetracycline, (66.6%) resistant to gentamicin, and (16.6% )resistant to cefotaxime. These findings are partially consistent with our results, where (85.7%) of *Pseudomonas spp*. isolates were resistant to ampicillin, (92.9%) resistant to tetracycline, and (92.9%) resistant to cefotaxime. Additionally, we found that (92.9%) of *Pseudomonas spp*. isolates were regularly sensitive to only gentamicin. *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. 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 [58] .The differences in the our results obtained in many studies shows that the patterns of microbial infection are not consistent in patients with skin infections; therefore, repeated evaluation of microbial characteristics and the antibiotic sensitivity is necessary for the selection of appropriate antibiotics [57].

In conclusion, the present study revealed that the most common isolates of skin infections were Gram positive bacteria , *S. aureus*, *S. epidermidis* and Gram negative bacteria were *P. aeruginosa*, *E. coli*. , the majority of Gram positive and Gram negative bacterial isolates were multidrug resistance. Therefore, the treatment of skin infections in our area after identified of bacterial pathogens must be guided by standard antibiotic susceptibility testing.

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