

Bacterial detection, identification, and isolation in Clinical Samples: A case of Tertiary Care Hospital in Mardan, KPK of Pakistan

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Abstract

Background: Bacteria are stunningly designed by Mother Nature and have the potential to challenge the most intellectual creature (human) in World. Pyogenic infections are characterized by the presence of local inflammation accompanied by the formation of pus and systemic symptoms. The bacteria involved are known as pyogenic, or pus-producing bacteria, and infections of soft tissue are typically accompanied by pus production. Infection of soft tissue and wounds may involve a wide range of aerobic species as well as anaerobic bacteria, either alone or in combination.

Objectives: The goal of the current research is to ascertain how often bacteria are found in blood and pus samples.

Study Design: Cross-Sectional Research

Material & Methods: Blood samples for culture were taken aseptically from veins up to 5 ml. Pus samples for cultures were aseptically collected in a sterile tube or disposable sterile syringe. All these samples were inoculated at 37 °C on appropriate culture media (Oxoid, MacConkey's agar, blood agar, chocolate agar) and incubated for 24 hours. For recording negative blood culture reports, 5 days' protocol was followed. Positive cultures were further proceeded by sub-cultures on blood agar, Mannitol Salt Agar (MSA), MacConkey agar, cholerae media, and spell to positivity was noted.

Results: Most common bacteria isolated from the blood cultures were *E. coli*, 28 %, *Coli* form 30 %, and *Citrobacteria* 15 % and followed by *Staphylococcus aureus* at 17 % and *Klebsiella* species at 10 % while the most common bacteria from the pus cultures were

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prevalent bacteria are staphylococcus aureus form 62 %, Coli at 15 %, Citrobacteria at 15 % and followed by. Klebsiella species 15 % and Citrobacteria.

Conclusion: The most frequently isolated bacteria from blood was E. coli, and Staphylococcus aureus was the most frequent germ recovered from pus samples.

Keywords: E. coli, Peshawar, Pus, Blood, Staphylococcus aureus

1 Introduction

The present-day lifestyle and less health awareness among individuals create numerous health problems (Al Halbusi et al. [2022](#); Jaffar [2020](#); Jaffar [2020](#); Mohammadi et al. [2021](#); Su et al. [2021](#)), including microbial infections, and metabolic disorders (Narayanan et al. [2021](#)). Bacteria are stunningly designed by Mother Nature and have the potential to challenge the most intellectual creature (human) in World (Doron S [2020](#)). Bacteria are very diverse and flexible environmental poses health issues and creatures and human pathogens (Iorember et al. [2022](#); Lebni et al. [2021](#); Shoib et al. [2022](#); Soroush et al. [2021](#); Su et al. [2021](#)). Bacteria may cause acute and chronic, deadly relapsing, community-acquired, or nosocomial infections (Winn [1995](#)). In recent years, vector-borne parasitic, as well as bacterial infections, have emerged or re-emerged in various geographical regions causing a global health and economic problems (Hafeez et al. [2023](#); Iorember et al. [2022](#); Local Burden of Disease [2021](#); Micah et al. [2023](#); Peter et al. [2017](#)), include humans, companion animals, livestock, and wildlife (Harrus and Baneth [2005](#)). The virulence of bacteria and their susceptibility to antibiotic drugs may be attributed to the molecular structure of the cell wall, periplasm, plasmid, cytoplasm, and membranes that separate these sections (Garg et al. [2003](#)). The skin and soft tissue infections (STI) caused by microbes during or after burn injuries, trauma, and surgical procedures pus, the composition of pus is a white to yellow fluid, dead WBCs, cellular debris, and sometimes necrotic tissues (Cogen et al., [2008](#)).

Pyogenic infections are described by systemic and local inflammation with pus formation that cause various health problems (Farzadfar et al. [2022](#); Moradi et al. [2021](#); Su et al. [2022](#); Su et al. [2021](#); Zafar et al. [2022](#)). Infections of soft tissue are usually coupled with the formation of pus and the bacteria involved are said to be pyogenic or pus-producing bacteria, which cause health issues (Azhar et al. [2018](#); Geng et al. [2022](#); NeJhaddadgar et al. [2022](#); Schmidt et al. [2022](#); Zeidabadi et al. [2022](#)). A wide-ranging variety of aerobic species, as well as anaerobic bacteria, may be present either alone or in a mixture in the infection of soft tissue and wound (Garau et al. [2013](#); Scalise et al. [2015](#); Verma et al. [2012](#)). The pus formation is a primary response to an infectious agent and mechanical injury to the tissues and other organs (Grace et al. [2020](#)). Complicated cutaneous and soft tissue infections are common and frequently need treatment in hospitals (Aqeel et al. [2021](#); Aqeel et al. [2021](#); Paulson et al. [2021](#); Toqeer et al. [2021](#); Yao et al. [2022](#)). They are a very heterogeneous group of infections for example abscesses, diabetic foot infections fasciitis, cellulitis, post-trauma, surgical site infection, as well as superficial abscesses in any anatomical site where the risk of anaerobic or Gram-negative bacteria involvement is high (Fattahi et al. [2020](#); Khazaie et al. [2021](#); Pouresmaeil et al. [2019](#); Su et al. [2021](#); Yoosefi Lebni et al. [2021](#)), for example, the rectal part. Colonization with the spreading of bacterial flora could lead to wound infection which can be severe and even occasionally leads to death (Bowler et al., [2001](#)). Bacteria, viruses, fungi, and protozoan cause wound infection. The common abscess-producing bacteria are Staphylococcus aureus, Pseudomonas, Klebsiella species, streptococcus, and Escherichia coli, while among all these bacteria Staphylococcus aureus is the most common bacteria that produce

pus furthermore may be poly-microbial and could involve both aerobic and anaerobic, Gram-negative and, Gram-positive bacteria (Moet et al. [2007](#); Talan et al. [2011](#); Rakesh Kumar [2013](#)). The emerging frequency of resistance to numerous antibiotics in bacteria makes SSTI progressively challenging to treat (Moet et al. [2007](#)). Moreover, the choice of management is often complicated by the need to treat without a confirmed diagnosis (Talan et al. [2011](#)). Methicillin-resistant *Staphylococcus aureus* (MRSA) is predominant in the majority of the nations where it is sought for. This is one of the most important bacteria in hospital-acquired infections (Parajuli et al., [2014](#)). The spread of multidrug-resistant (MDR) bacterial pathogens has further a new angle to the problem of wound infections. (Jain et al, [2015](#); Kotz et al. [2009](#)).

Bacteremia is the existence of bacteria in the lifeblood, an incident of the occurrence of bacterial colonization in blood circulation is typically not a life-threatening state (Azizi et al. [2021](#); Jaffar et al. [2019](#); Lebni et al. [2020](#); Moradi et al. [2020](#); Su et al. [2020](#)). Bloodstream infections (BSIs) are closely associated with significant patient morbidity, and mortality and are amongst the utmost common healthcare infections (Abbas 2021; Maqsood et al. [2021](#); NeJhaddadgar et al. [2020](#); Shuja et al. [2020](#); Yoosefi Lebni et al. [2020](#)). BSI is a serious problem that needs immediate attention and treatment, particularly if caused by MDR organisms (Jaffar et al. [2022](#)). It is valued that two million patients each year in the United States (US) acquire infections although, in hospitals, roughly 350,000 of these infections include the BSIs, and around 90,000 are deadly (Diekema et al, [2003](#)). A wide range of microorganisms especially bacteria can cause BSIs, nevertheless, many of these bacteria are also part of normal flora (Saha et al., [2016](#)).

A Widespread range of multidrug resistance bacteria can be caused blood infections that can lead to more morbidity, motility, and complications in hospitalized persons (Keihanian et al. [2018](#)). The common BSI bacteria are streptococci, enterococci, Enterobacteriaceae, or staphylococci (Tiwari MK [2021](#)). BSIs can cause local infection of organs or tissue like pneumonia or meningitis, or they can cause generalized infection such as septicemia without any localization (Brown et al. 2005). The frequency of drug resistance among the organism that causes BSIs is getting amplified bit by bit alarmingly, hence rendering common infections also more challenging to treat or untreatable, resulting in disturbing concerns to patients.

The discovery of antibiotics unlocked a new era in the management of infectious diseases but regular administration of drug formulation produces drug resistance among the microorganism (Narayanan et al. [2021](#)). Drug resistance especially antibiotic resistance has been denoted as “The silent tsunami facing modern medicine” (Nagvekar et al. [2020](#)). Meanwhile, the microorganisms efficiently get the genomic level evolution and develop resistance to medicines such as antibiotics. The research community, broadcasting, as well as political inventiveness, are creating awareness about drug resistance. Despite this hard work and awareness, antibiotic resistance is continuously snowballing all over the world (Exner et al. [2017](#)). According to the World Health Organization (WHO) fact sheet report, infections through antimicrobial-resistant organisms can upshoot in failure of cure, an increased charge of treatment, increased hospitalization stay, as well as an increased socioeconomic burden. In past, diseases caused by MDR Gram-negative bacteria have intensely increased across the world.

The preferred antibiotic resistance mechanisms in bacteria include modifications to antibacterial target proteins on cell surfaces, changes in membrane permeability, and drug degradation through

enzymatic activity. (Mathiyazhagan et al, [2013](#); Narayanan et al, [2020](#)). All these collective resistance mechanisms could intercede through chromosomes and plasmids (Narayanan, et al. [2020](#)). Penicillin-resistant A good illustration of how the global movement of people spreads multi-drug resistant bacteria is streptococcus pneumoniae (PRSP). The DNA from penicillin-resistant commensal streptococci is transformed into penicillin-resistant commensal streptococci, with plasmids serving as the main vector in most bacteria, primarily Gram-negative bacteria. The importance of plasmids in Shigella bacteria that carry multiple drug resistance (MDR) indicators. Despite the fact that Escherichia coli (E.coli) was initially identified over 40 years ago in Japan (Phan et al. [2009](#), Gould [2008](#)). The synthesis of β -lactamases enzymes by Gram-negative bacteria is the main method of antibiotic resistance. (Hawkey et al, [2009](#)). MDR microorganisms are estimated to cause 10 million deaths by 2050 and roughly 700,000 diseases worldwide each year. They also severely harm economic output. (Martín et al, [2014](#); Gogoi et al, [2021](#)). MDR bacteria, such as vancomycin-resistant enterococci (VRE) and methicillin-resistant Staphylococcus aureus (MRSA) have been the subject of several research investigations due to their increasing resistance over time. Contrarily, Pseudomonas aeruginosa, Klebsiella species, Enterobacter species, Acinetobacter baumannii, and Escherichia coli are some bacteria that are showing a growing trend of antibiotic resistance. (Gaynes et al, [2005](#);Gould [2008](#)).

2 Materials and Methods

The current investigation was conducted between January and August of 2022 and followed research protocol (Al-Sulaiti [2022](#); Al-Sulaiti et al, [2004](#); Li et al, [2022](#); Shah et al, [2023](#); Zhuang et al, [2022](#)). The research was conducted at Bacha Khan Medical College Mardan (BKMC), situated in the Mardan district of Khyber-Pakhtunkhwa (KPK), Pakistan. All the patients who had come for blood culturing and pus culturing were included in this study, and skin commensals and contaminants were not included in the study.

Laboratory data collection: From outpatient and inpatient departments, 778 clinical blood and urine samples were obtained. Clinical samples were processed at the Microbiology lab, Pediatric patients who received antibiotics within the past 2 weeks were excluded.

Isolation and documentation of Bacteria: Blood samples for culture were taken aseptically from veins up to 5 ml. Pus samples for cultures were aseptically collected in a sterile tube or disposable sterile syringe. All these samples were inoculated at 37 °C on appropriate culture media (blood agar, MacConkey's agar, chocolate agar, Oxoid) and incubated for 24 hours. For recording negative blood culture reports, 5 days' protocol was followed. Positive cultures were further proceeded by sub-cultures on MacConkey agar, blood agar, Mannitol Salt Agar (MSA), cholerae media, and spell to positivity was noted.

Antimicrobial susceptibility: All positive + cultures were further processed for identification of pathogens, gram staining, and biochemical tests (Gram's staining, appearance, morphology, catalase, oxidase reaction, motility, triple sugar iron (TSI), urease test, citrate test, DNase test, indole test.

Statistical Analysis: The Statistical Analysis for the Social Sciences (SPSS) version 26 was used for the assessment of the research study's data collection. The outcomes of these statistical analyses were then put through more processing in Microsoft Excel 2011.

3 Results and Discussion

A total of n = 819 patients for blood and pus culturing visited the BKMC Microbiology laboratory. Out of these 778 patients, 321 (41 %) individuals were tested for blood culturing, and 457 (59%) patients were tested for pus culturing. Among 321 blood cultures, 26 were positive and the overall incidence of bacteremia was 44.13%. A total of n=457 samples were recruited in the BKMC

Microbiology lab. Out of n=samples, 266 samples were negative and n=samples were positive, the detail as shown in Figure/Table: 1. this current finding is very similar to a study conducted on the geographical distribution of bacteremia it was reported that in India 16%–44 % and in Iran as 4%–18% and Iran as 4%–18%. (Keihanian et al, [2018](#)). The current results are contrasted on the frequency of blood isolate for bacteremia in different geographical regions, reported the most prevalent bacteria from blood cultures was as follows: *Pseudomonas aeruginosa* 29.3 %) coagulase-negative *Staphylococcus* and then followed by *Pseudomonas aeruginosa* 29.3 % (Keihanian et al, [2018](#); Saeidinia et al. [2016](#)). According to another study, the most prevalent bacterium was *Staphylococcus* spp. isolated from skin or soft tissues (n = 197 samples), which was followed by *E. coli*, which was isolated from urine (n = 646 samples). Bacterial species did not spread uniformly across the nation. 95.7% (n = 1,919 samples) of isolates had consistent bacterial species identification using all methods. 2022) (Rebelo et al. Similar findings indicated that *E. coli*, *Streptococcus uberis*, and coagulase-positive staphylococci were the most frequently isolated significant pathogens. (Green et al. [2005](#)).

Table No: 1.1 Frequency of Blood and Pus Cultures in Clinical Isolates

Culture Type	Number of specimens	Negative	Positive
Blood culture	321	274	47
Pus culture	457	266	191
Total	778	69 %	30 %

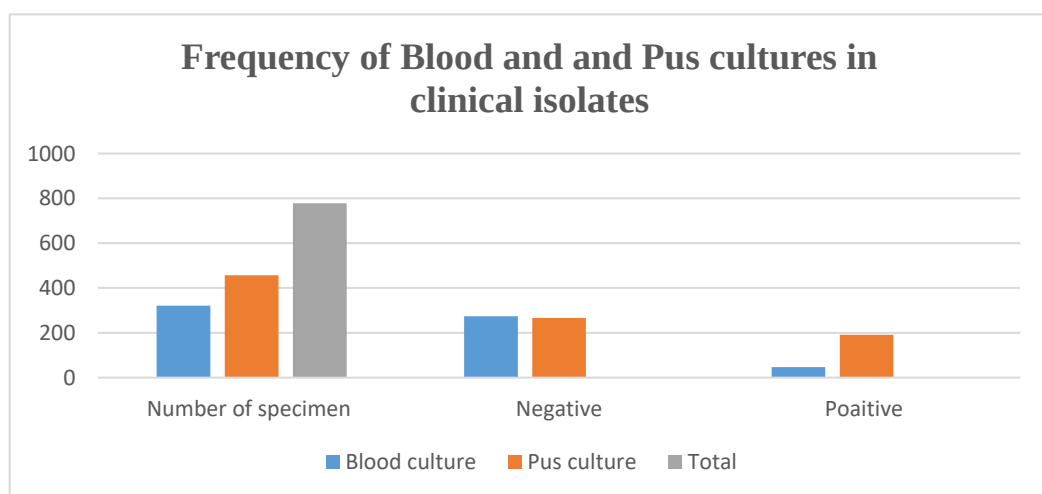


Figure No: 1.1 Frequency of Blood and Pus Cultures in Clinical Isolates

In this investigation, 321 samples in total were cultivated, and 47 samples yielded favorable results. From blood cultures, six different kinds of bacteria were identified. Among different bacteria the most prevalent bacteria are *E. coli* form 28 %, *Coli* forms 30 % and *Citrobacteria* 15 % and followed by *Staphylococcus aureus* 17 %, and *klebsiella* species 10 %, the detail of all different bacteria is shown in Figure/Table No 2. This study's findings are quite similar to those of a study done in India, where the prevalence of main bacteria isolated was reported to be 24% for *Staphylococcus aureus*, 20% for *E. coli*, followed by 16% for *Staphylococcus epidermidis* and *Streptococcus* spp, and 10% for *Klebsiella* spp. (Sumathi,et al, [2008](#)). The high frequency of *Staphylococcus* sp. followed by *E. coli* in the current study, according to another study, is consistent with the work of many other researchers. The most significant and common bacteria causing mastitis worldwide are staphylococci (Unakal et al, [2010](#)). As opposed to a study on the

percentage of Enterobacteriaceae, and Klebsiella spp. in particular, dropped over time, the next most frequently isolated bacterial species after E. coli were Streptococcus spp., Klebsiella spp., Enterococcus spp., Staphylococcus spp., and Enterobacter spp. Separate cultures of Enterococcus spp. have been performed more frequently lately (Unakal et al, [2010](#)). In a study on bacterial isolates from blood cultures of children in Nigeria with probable septicemia, Staphylococcus aureus was shown to be the most common isolate (48.7%), followed by Coliforms (23.4%) (Meremikwu et al. [2005](#)).

Table No: 2.2: Frequency of different bacteria in Blood Cultures

Number of Bacteria (47)	Bacterial Isolates names	Percentage %
8	Staphylococcus aureus	17
5	Klebsiella Species	10
13	Coli form	28
14	E.coli	30
7	Citrobactor Species	15

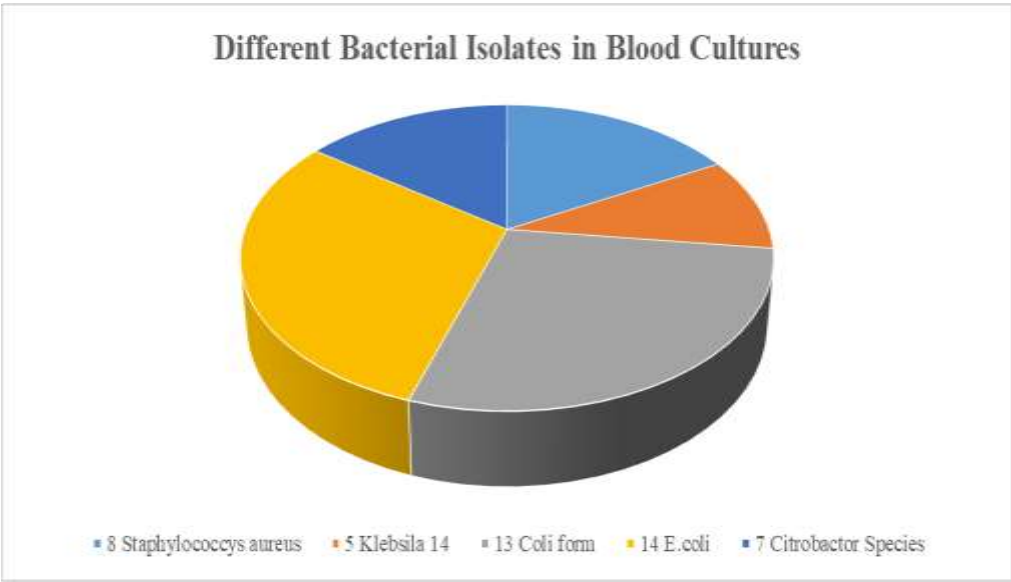


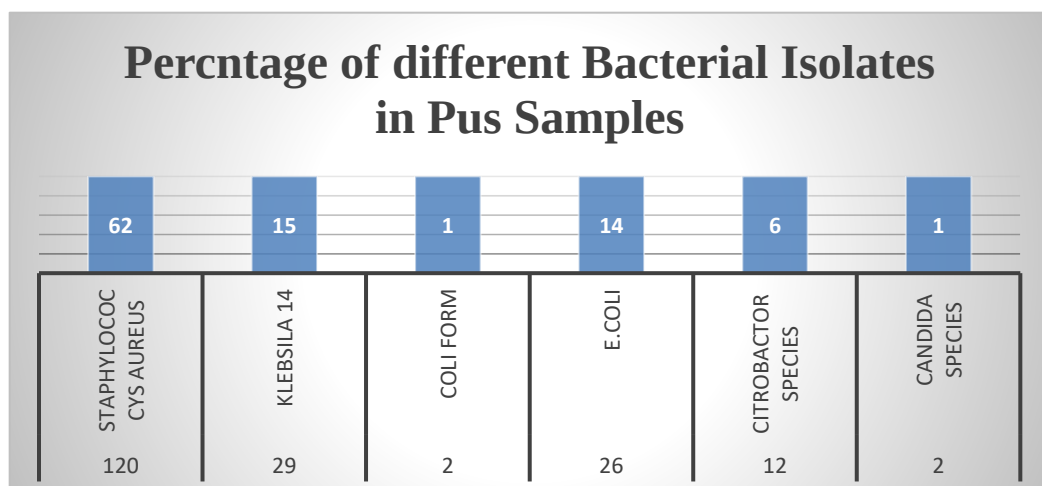
Figure No: 2.2: Frequency of different bacteria in Blood Cultures

In this investigation, a total of 457 samples of pus were cultured, and 191 of those samples tested positive for the infection. From pus cultures, six different bacteria were recovered. Staphylococcus aureus makes up 62% of all bacteria, followed by Coli 15% and Citrobacteria 15% as the most common bacteria. The breakdown of all the various bacteria is illustrated in Figure/Table No. 3, with Klebsiella species accounting for 15% of the total. According to a 2011 study by M et al, P.

aeruginosa is one of the most important pyogenic bacteria and the third most common reason for pyogenic infections, with variable prevalence depending on the geographic region and the clinical situation. Pyogenic nosocomial bacterial infections are closely associated with *P. aeruginosa*. (MM et al, [2021](#)). A similar study reported that the most predominant bacteria in pus samples is a staphylococcus aureus (Zafar et al, [2012](#)). Another similar study reported that bacterial species which were identified in our study as compared to the given finding: Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus spp., E. coli, Proteus vulgaris, Micrococcus luteus, Bacillus cereus (Agri 2011). The Mardan investigation, according to KPK, found that E. coli made up the majority of the isolates (90.3%), followed by Pseudomonas aeruginosa (7.2%), Staphylococcus aureus (1.3%), and Klebsiella pneumonia (1.3%). (Sajid Ali et al. [2021](#)).

Number of Bacteria (191)	Bacterial Isolates names	Percentage %
120	Staphylococcus aureus	62
29	Klebsiella 14	15
2	Coli form	1
26	E.coli	14
12	Citrobactor Species	6
2	Candida Species	1

Figure No: 2.3: Frequency of different bacteria in Pus Cultures



Conclusion: E. coli was the most typical bacteria to be isolated from blood cultures, 28 %, Coli form 30 %, and Citrobacteria 15 % and followed by. Staphylococcus aureus 17 %, and klebsiella species 10 % while the most common bacteria from the pus cultures were prevalent bacteria are staphylococcus aureus form 62 %, Coli forms 15 % and Citrobacteria 15 % and followed by. Klebsiella species 15 % and Citrobacteria.

Conflict of Interest: The author has no conflict of interest.

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