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Evaluation of Antimicrobial Susceptibility Patterns of Bacteria in Pus Samples of Last Three Years at Chaghi Laboratory, Quetta, Pakistan

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Abstract

Antibiotic resistance develops and spreads among pyogenic bacteria as a result of injudicious antibiotic usage in the treatment of pyogenic illnesses. Significant gaps in research on antibiotic resistance in Quetta, Pakistan have been observed making it difficult to develop trend lines and resistance patterns that can contribute to fighting the dangers associated with the rise of antimicrobial resistance. The objective of this study was to determine the antibiotic susceptibility patterns of bacterial isolates obtained from pus samples. A retrospective analysis was carried out on regular pus specimens collected at the Chaghi laboratory in Quetta from January 2018 to December 2020. A total of 259 specimens were examined for antibiotic susceptibility patterns, with Ciprofloxacin (85.7 %), Cephradine (78.4 %), Ofloxacin (77.6 %), and Augmentin (56%) having the greatest proportion of resistance. All *S. aureus* strains were vancomycin-sensitive (100%) and Ampicillin-resistant (98.6%), while all gram-negative bacteria were sensitive to Imipenem (97.9%) and Tazobactam (90%) however resistant to Ampicillin (98.6%). A gradual increase in antibiotic resistance was observed among gram-negative isolates and a notable increase in resistance was observed by *S. aureus.* S. aureus was shown to be the most frequent bacteria in pus samples, followed *by K. pneumoniae*, *E. coli*, and *P. aeruginosa*. We concluded that antibiotic overuse should be avoided to prevent emerging resistance. Regular surveillance of antibiotic susceptibility patterns also aids in the development of improved treatment options for reducing morbidity and death by recognizing the real burden of antibiotic resistance in an organism and preventing its spread.

Keywords: Pyogenic Infection, Antibiotic resistance, S. aureus, *K. pneumoniae*, *E. coli*, and *P. aeruginosa*

Introduction

Infectious illnesses caused by microbes have a catastrophic influence on human health. Since the discovery of penicillin by Alexander Fleming in 1928, antibiotic medicines have aided in the treatment of human bacterial illnesses. Its use in the 1940s ushered in the emergence and widespread use of conventional antibiotics in medicine (Anonymous, 2020). Because of their higher efficacy and selectivity, these medicines were preferred over natural chemicals in the treatment of infectious illnesses (Thormar, 2011). However, due to selection pressure on susceptible strains causing resistant strains to survive, their extensive use in preventing and treating a human, animal, and plant diseases resulted in the establishment and spread of antibiotic resistance (Bell et al., 2014).

Antibiotic resistance (AR) is the potential of pathogenic bacteria to withstand the effects of antibiotic drugs and can be caused by natural or acquired (Nolte, 2014). Erroneous diagnosis, illogical antibiotic usage, and irregular antibiotic intake, potentially owing to an incorrect prescription or poor compliance, are all factors that lead to antibiotic resistance. As a result, addressing these factors can help to prevent antibiotic resistance from spreading (Chamoun et al., 2016). Local and systemic inflammation, generally with pus production, characterizes pyrogenic infections (Koneman, 1999). These might be either endogenous or exogenous in nature. Surface bacteria can get access to the skin through a breach in the skin, grown locally. Immune cells are brought into the region to combat pathogens as part of the body's defensive system. The buildup of these cells eventually results in pus, a thick white substance (Chopra et al., 1994).

Antibiotic resistance pathogenic microorganisms have emerged as a danger to public health around the world (Rice 2006). Gram-negative bacterial strains such as *E. coli, K. pneumoniae, P. aeruginosa*, and Gram-positive *Staphylococcus aureus* have become increasingly associated with pus infections in hospital settings due to widespread antibiotic overuse and insufficient dose regimens (Iredell, 2016; Rice, 2006). Various studies have been done from time to time all over the world to evaluate the bacterial profile and antibiotic susceptibility pattern in pus samples. This is especially important for the treating physician, who must begin empirical therapy of the patient while waiting for lab culture results (Ramesh et al., 2014). Though the bacterial profile from pus samples is consistent across studies, there is significant fluctuation in the antibiotic susceptibility pattern of these isolates, emphasizing the growing concern of the evolution of resistant bacteria and the necessity for ongoing monitoring of such changes.

Antibiotic resistance to frequently prescribed drugs has emerged at an alarming rate in Pakistan and negligence towards prevention and control practices, surveillance, and unreported data has been one of the biggest contributions towards the current scenario of AR in Pakistan and same goes for Baluchistan, respectively. Significant gaps in research on antibiotic resistance in Quetta have been observed due to a lack of studies on bacteria causing pyogenic illnesses, making it difficult to develop trendlines and resistance patterns that can contribute to fighting the dangers associated with the rise of AMR in Quetta. To investigate the shifting patterns in antibiotic resistance in different pus isolates, a study was undertaken at Chaghi laboratory Quetta.

The current Retrospective study is the first effort to describe the antibiotic-resistance scenario in Quetta from pus samples of the last three years undertaken at Chaghi laboratory Quetta. It aims to find the gaps in surveillances and recommendations for researchers and prescribers founded on the outcomes. To identify the overall percentages of bacterial isolates and among both gender patients in pus samples. To evaluate the antibiotic susceptibility patterns of gram positive and negative bacteria in the pus samples. To determine the overall potency of antibiotics on bacterial isolates during the study period.

Materials and Methods

Chaghi Laboratory is a private laboratory located near City International Hospital Zarghun Road, Quetta. The Chaghi laboratory performs several microbiological diagnostic tests such as gram staining, media culturing, and antibiotic susceptibility tests. Located in the proximity of several big hospitals of Quetta with a greater influx of patients and recorded valid data was the choice of selection of laboratory for study.

Data collection

The diagnostic reports on all isolated organisms at the Chaghi laboratory for three years, from 2018 to 2020, were used in this retrospective analysis. For bacteriological analysis, the laboratory gets a variety of specimens. Bacteriological analysis entails the culture of specimens on suitable culturing media and antibiotic susceptibility testing. Microsoft Excel spreadsheet reports on Antibiotic Susceptibility tests (ASTs) performed on all isolated organisms from 2018 to 2020 were analyzed.

Inclusion and exclusion criteria

This research includes all specimen entries with information on the patient's gender, kind of specimen, isolated organism, and ASTs conducted. Entries that lacked any of the above-mentioned details, as well as specimens with uncertain specimen types and specimens with no growth, were excluded from the research.

Data analysis

Microsoft Excel 2016 and IBM SPSS Statistics version 20 were used to analyse the AST findings. Applying percentages and cross tabs, the rates of antibiotic susceptibility were determined for each bacterial isolate separately, by patient's gender, and year of sample processing. The number of resistant/susceptible strains out of the total number of strains exposed to a specific antibiotic in a specimen was used to determine the mean percentages of susceptibility of each isolate to all tested antibiotics.

Results & Discussion

Overall Percentages of Bacterial Isolates among Both Gender Patients in Pus Samples

Antibiotic resistance (AR) has recently gained attention in clinical settings across the world because of its implications on rising healthcare expenditures, morbidity, and death among patients suffering from infectious illnesses. The problem is exacerbated in underdeveloped nations since information on antibiotic susceptibility patterns of bacterial isolates is often inconsistent (Hart, & Kariuki, 1998). The present Retrospective research is the first attempt to understand the antibiotic-resistance scenario in Quetta isolated from pus samples in previous three years. Our first objective was to identify the overall percentages of bacterial isolates and among both gender patients in pus samples. A total of 353 pus samples were analyzed and only 259 (73%) met the inclusion criteria for this study. These 259 samples had growth of organisms, which were tested for sensitivity pattern by standard laboratory methods, the remaining 90 cases were culture negative. Among the culture grown cases, 118 (46%) samples were gram-negative and 141 (54%) were gram-positive organisms as depicted in **Figure 1**. In our study, of the 353 cases sent, 73% were culture positive compared to 76% by Savanur *et al.* (2019) among these, gram-negative accounted for 46% and gram-positive was 54% comparable to a study conducted by Rai *et.al* (2017) where similarly gram-positive bacteria were mostly isolated from pus samples. S. aureus (54.4%) was the most prevalent isolate among Gram-positive bacteria found in these samples, which is consistent with earlier research (Lakhey & Bhatt 2007; Acharya et al., 2008; Kumari et al., 2018). Among the gram-negative pathogens encountered, Klebsiella pneumoniae was the most common pathogen with 18.9% occurrence, followed by E. coli (18.1%) and Pseudomonas aeruginosa (8.5%) (**Figure 2**), which contrasts with other studies conducted by (Trojan 2016; Trouillet 1998; Basu, 2009) where E. coli was the most common isolate followed by Pseudomonas. The isolates between male and female patients was almost equal **(Figure 3).** Male and female patients had modest variations in the frequency of bacterial species.

Antibiotic Susceptibility Patterns of Gram-Negative and Gram-Positive Bacteria

Pyogenic infections are distinguished by local and systemic inflammation, which is frequently accompanied by pus formation. Gram-negative bacteria like *Pseudomonas*, *E. coli*, *Klebsiella spp.*, *Proteus spp.*, and Gram-positive cocci like

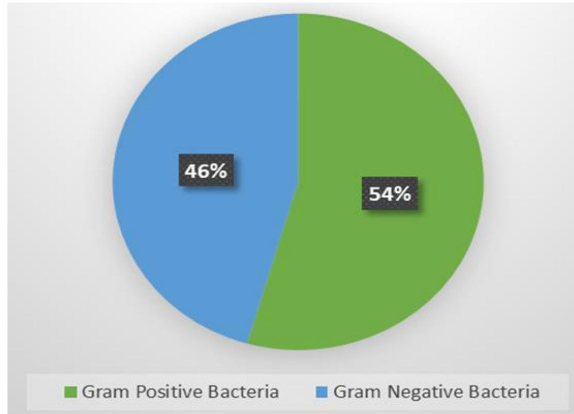

Figure 1. Percentages of gram-positive and gram-negative microorganism.

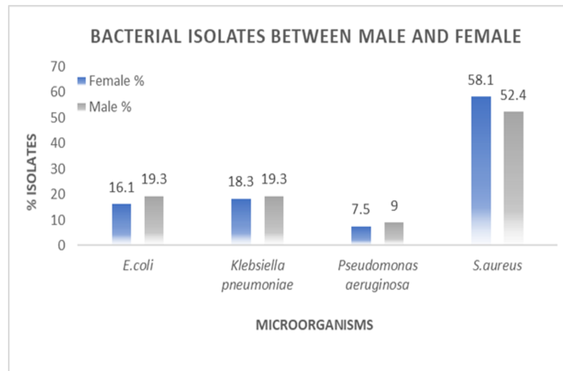

Figure 2. Percentages of bacterial isolates between both genders.

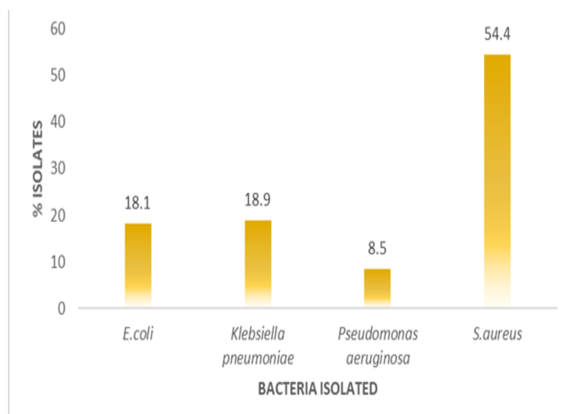

Figure 3. The overall percentages of bacteria observed.

Staphylococcus aureus and *Enterococci* are the most common pathogens of pus (Thangavel 2017). The study also evaluated the antibiotic susceptibility patterns of gram positive and negative bacteria in the pus samples. According to the results *E. coli*, *K. pneumoniae*, *P. aeruginosa* showed the highest resistance to Beta lactam (94.4%), followed by Fluoroquinolones (85.5%) and Cephalosporins (82%) (**Figure 4**). However, *E. coli*, *K. pneumoniae*, *P.*

aeruginosa were most sensitive to Imipenem (97.9%), followed by Tazobactam (90.0%) and Amikacin (81.5%) (**Figure 5**). The susceptibility pattern of *S. aureus* exhibited the highest resistance to Ampicillin (98.6%) followed by fluoroquinolones (87.6%), erythromycin (81.6%) and Cephradine (80.9%)

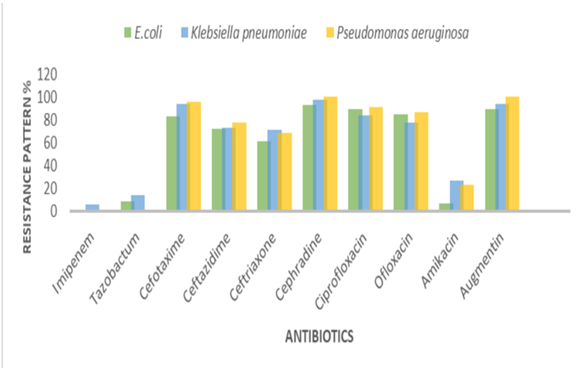

Figure 2.Antibiotic resistance pattern of Gram negative isolates.

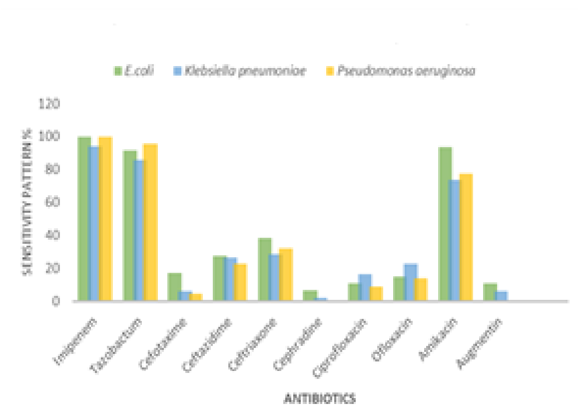

Figure 5. Antibiotic sensitivity pattern of Gram negative isolates.

(**Figure 6**). *Staphylococcus aureus* was most sensitive to Vancomycin (100%), followed by Fusidic acid (93.6%) and Gentamicin (85.8%) (**Figure 7**). Beta lactam (94.4%), fluoroquinolones (85.5%), and Cephalosporins (85.5%) showed the most resistance among Gram-negative isolates (82.3%). This might be due to an increase in extended spectrum beta-lactamases (ESBL) expression among gram-negative bacteria. The rates of antimicrobial resistance to Augmentin and Cephalosporins have been steadily increasing over time might be attributed to doctors avoiding the extremely resistant amoxicillin and penicillin. Our study also found an increase in resistance to 3rd generation Cephalosporins (e.g., ceftriaxone), which can be linked to the expansion of extended range beta-lactamase generating bacteria (Kim et al., 2017). Antibiotic resistance has become widespread, particularly among gram-negative bacteria, as a result of the widespread usage of antibiotics (Ahmed et al., 2003). Bacteria can develop resistance and pass it along from one bacterium to the next (Krishnamurthy 2016). Drug-resistant illnesses might accelerate the spread of new infectious diseases (Paul 2017). Antimicrobial resistance has become widespread among bacterial infections, making management and treatment challenging (Ananthi 2017).

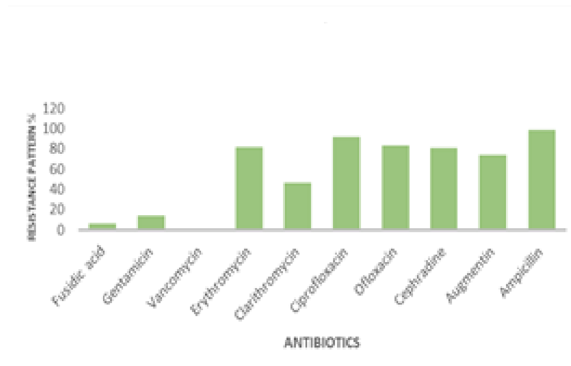

Figure 6. Antibiotic resistance pattern of S.aureus demonstrating majorly resistance developed against Ampicillin (98.6%) followed by fluoroquinolones (87.6%), erythromycin (81.6%) and Cephradine (80.9%).

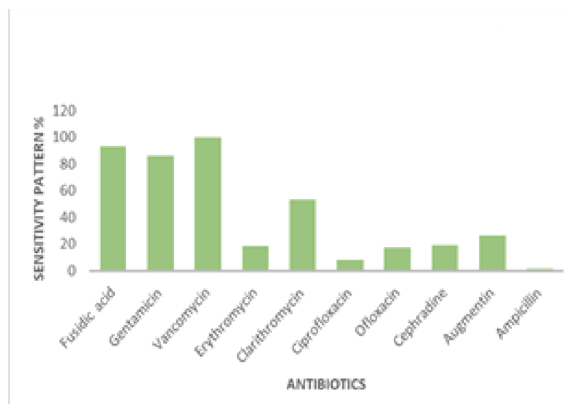

Figure 7. The antibiotic sensitivity pattern of Staphylococcus aureus illustrating the most sensitive antibiotics for treatment including Vancomycin (100%), followed by Fusidic acid (93.6%) and Gentamicin (85.8%).

Overall Potency of Antibiotics on Isolated Bacteria

We determined the overall potency of antibiotics on bacterial isolates during the study period. Generally, isolates were mostly resistant to Ciprofloxacin (85.7%), Cephradine (78.4%), Ofloxacin (77.6%), Augmentin (56%), while Vancomycin (54.4%), Fusidic acid (50.6%) and Gentamicin (45.9%) retained their effectiveness against pus producing bacteria (**Figure 8**). Bacteria, particularly *Enterobacteriaceae*, generate beta-lactamases, which hydrolyse the amide bond of antibiotics like penicillin and amoxicillin's four-membered distinctive ß-lactam ring, leaving them ineffective against infections. Our findings show a statistically significant rise in the frequency of *Enterobacteriaceae* that produce extended-spectrum Beta-lactamase (Pitout, 2010). Multi-drug resistant bacteria contribute to the high rates of AMR to ß-lactams (e.g., penicillin, amoxicillin, and ceftriaxone) by Gram-negative bacteria in this study. Antibiotics that function as weak labile inducers (e.g., ampicillin and other ß-lactams) are clinically unsuitable for treating ESBL-producing bacterial infections (Piccirillo 2001).

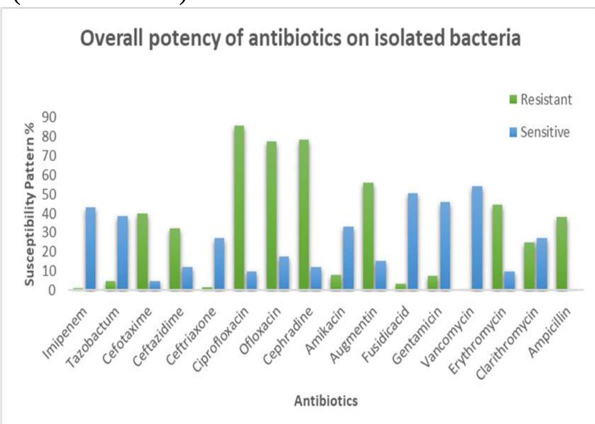

Figure 8.The overall potency of the antibiotics on isolated bacteria including Gram negative and Gram positive during last three years.

According to the study findings, carbapenem resistance by Enterobacteriaceae was very low (2%) during the three years of this investigation. The findings are similar to the findings of Magwenzi *et al.* (2017), who found a 1% incidence of carbapenem-resistant *Enterobacteriaceae* in Harare. One remaining treatment option for ESBL-producing Enterobacteriaceae is the use of carbapenems (i.e., imipenem, ertapenem, meropenem and doripenem). In terms of the sensitivity pattern of the gram-negative isolates, it was discovered that Imipenem (98%), Tazobactam (91%), and Amikacin (81.4%) were the most effective drugs. These findings were consistent with the findings of earlier research by Rao *et al.*, (2014) and Amatya *et al*., (2015). *S. aureus* is one of the major causes of wound infections, ear infections, and brain abscesses in hospitals, and its prevalence has grown in recent decades, which is consistent with the findings of our investigation (Espersen, 1995). *S. aureus* exhibited high antibiotic resistance to Ampicillin (98.6 %), Fluoroquinolones (88 %), Erythromycin (82 %), and Cephradine (81 %). These findings are comparable to those of Rao *et al* (2014), who discovered that *S. aureus* is resistant to penicillin (84.62 %) and erythromycin (84.62%). In the current study, the most effective antibiotic against *Staphylococcus aureus* was found to be vancomycin (100%) followed by Fusidic Acid (94%) and Gentamicin (86%). Some other studies showed a similar result for *Staphylococcus aureus* with 100% sensitivity against vancomycin (Anwar *et al*., 2003; Bhatt *et al*., 2014). In other studies, Fusidic acid (88.5%) showed followed by Gentamicin (83%) antimicrobial susceptibility which supports the result of our study (Ahmed *et al*., 2003; Bhatt *et al*., 2014).

Resistance Pattern in the Past Three Years

The last objective of the study was to determine the antibiotic resistance pattern of bacteria isolated from pus samples in the past three years. Over the study period, gram-negative bacteria showed a modest development in antibiotic resistance, whereas S. aureus exhibited a statistically notable increase in resistance (**Figure 9**). As antibiotic resistance among microorganisms grows, it is becoming increasingly important to choose antibiotics carefully and to deliver them at the right dosage and time. In our investigation, we discovered that E. coli, S. aureus, K. pneumoniae,

Figure 9. Antibiotic resistance pattern of both Gram negative and Gram positive bacteria during last three years.

and P. aeruginosa isolates from pus had significant drug resistance to various antibiotics. As a result, appropriate antibiotic policy and infection control measures must be deemed vital. Knowledge of the bacteriology of an illness, as well as laboratory susceptibility testing of the microorganisms involved, may help to rationalize medication selection in antimicrobial chemotherapy34. Several studies have found that antibiotic resistance to commonly given medicines is increasing at an alarming pace throughout Pakistan, especially Balochistan. However, it is worth noting that some significant variables that promote the spread of antibiotic resistance include the overuse/misuse of antibiotics as a result of factors such as erroneous diagnosis, and irrational antibiotic use accounting for nearly half of all antibiotic prescriptions for patients (Chamoun, 2016; Hart, 1998). Surveillance is a key component of offering a method for AMR management and prevention. Significant gaps in research on antibiotic resistance in Quetta have been observed due to a lack of studies on bacteria causing pyogenic illnesses, making it difficult to develop trendlines and resistance patterns that can contribute to fighting the dangers associated with the rise of AMR in Quetta.

Conclusion

In this study gram-positive bacteria were found to be more prevalent than gram-negative bacteria. The most frequent organism found in pus, is *S. aureus*, *Klebsiella spp.*, *Pseudomonas aeruginosa* and *E. coli*. Augmentin (94.4 %) was the least effective antibiotic against gram-negative bacteria, whereas Ampicillin (98.6 %) was the least effective antibiotic against gram-positive bacteria. Antibiotics like imipenem (97.9%) and tazobactam (90%) were the most effective against gram-negative bacteria. Antibiotics such as Vancomycin (100%) and Fusidic acid (93.6%) were extremely efficient against gram-positive bacteria. In underdeveloped nations, evidence-based practice is still in its initial developmental phase. Antibiotics are administered without doing laboratory tests for determining the etiologic agent and antibiotic susceptibility testing. Similarly, numerous medicines are readily available over the counter in a variety of pharmacies, raising the danger of growing antibiotic resistance. As a result, there is an urgent need to combat the spread of antibiotic resistance, particularly in developing countries like Pakistan, by implementing evidence-based antibiotic therapy through increased access to diagnostic laboratories, rational antibiotic use in hospitals, strengthening AR surveillance programs, and educating the public, clinicians, pharmacists on the prophylactic use of antibiotics.

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