Research Article

Emerging Resistance in Gram Negative Bacteria Isolated from Clinical Samples in Lahore, Pakistan: A Current Epilogue

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Abstract | Antibiotic resistance occurs when bacteria develop number of mechanisms to protect themselves against the action of antibiotics. Antibiotics are chemical substances which bacteria and fungi produce either to inhibit or kill the other microbes. Antibiotics are classified on the basis of their structure and mode of action. Bacteria resist antibiotics by inactivating the drugs, altering the target site, chemically modifying the antibiotics and by ribosomal splitting. Antibiotic resistance can be intrinsic or extrinsic. Bacteria transfer resistance to other microbes through conjugation, transformation and transduction. The present study was conducted at Ganga Ram Hospital Lahore in order to study the resistance pattern of gram-negative bacteria. Females are more prone to infections as compared to males due to anatomical, physiological, behavioral and hormonal imbalance that's why majority of samples (33/60) were obtained from females as compared to males (27/60). From these collected samples exceeding numbers were of blood samples (20/60) followed by urine samples (18/60), pus samples (12/60) and (10/60) samples were of sputum. Bacterial isolates contained higher numbers of *E.coli* (30/60), followed by *Pseudomonas aeruginosa* (13/60), then *Klebsiella* (12/60) and minimum number was of *Proteus spp.* (5/60). Family *Enterobacteriaceae* and *Pseudomonas aeruginosa* are responsible for infections of brain, eyes, ears, digestive tract, respiratory tract and urinary tract. It has become difficult to treat these infections as these microbes have become resistant to variety of antibiotics. Due to multidrug resistance only few choices are left to treat the bacterial infections.

1. Introduction

Resistance in bacteria is rising to dangerously high levels all over the world. New resistance mechanisms are emerging, we are heading for a post-antibiotic era, in which common infections and minor injuries can once again kill (Scott *et al.*, 2016). Antibiotics have played a vital role in medicine and surgery and have saved billions of lives all around the world (Rossolini *et al.*, 2014). Benefits of antibiotics are limited as compared to harmful effects due to increasing level of resistance in microbes against all

available antibiotics which is an alarming situation (Ventola, 2015).

Intrinsic resistance (Blair *et al.*, 2015) and acquired resistance, on the other hand result in horizontal gene transfer (Magiorakos *et al.*, 2012). Direct transfer of DNA through transformation (Hu *et al.*, 2017) leads to chronic infections because heavy dose is required to treat infection (Ribeiro *et al.*, 2016). ß-lactam drugs occupy a special place among antibiotics. Beyond the relative ease of dosing them and their usually good or acceptable tissue distribution (Gootz, 2010). Most important causative agent of urinary tract infection is *E. coli* (Gupta *et al.*, 2011) due to considerable resistance against variety of antibiotics like tetracycline, ampicillin, fluoroquinolones and cephalosporin (Welch, 2017).

Klebsiella is one of the prominent Genus of *Enterobacteriaceae* among them *K. pneumoniae* is the most prominent microbe (Nizet and Klein, 2011) responsible for high rate of causalities (Perez and Van Duin, 2013). *P.mirabilis* and *P.vulgaris* has stamina of transformation throughout life (Drzewiecka, 2016). Presence of enzymes AmpC and β lactamases (Bush and Jacoby, 2010). Several studies have revealed that *P. mirabilis* that produce AmpC β-lactamases and ESBL can cause outbreaks in developing and developed countries (Nakano *et al.*, 2012). Even amino glycosides, carbapenems, cephalosporins and fluoroquinolones resistant strains are reported from different countries which is an alarming situation (Moradali *et al.*, 2017).

1.1 Aims of study
Isolation and identification of infection causing bacteria. Detection of antibiotic resistant bacteria with antibiotic susceptibility profile. Characterization of bacteria with morphological and biochemical tests.

2. Materials and Methods

The present study was conducted at Sir Ganga Ram Hospital in order to study the resistance pattern in gram-negative bacteria.

2.1 Collection of clinical samples
Total 60 clinical samples of blood, urine, pus and sputum were obtained from patients. These samples were collected in sterile containers in order to prevent contaminations. Number, date and time

were mentioned at containers to prevent mixing of samples. Media plates of Blood agar, nutrient agar, MacConkey agar and agar were prepared to grow bacteria on them. Before preparation of these media, all the provisions were kept at incubator to get sterility. Sputum and Pus samples were cultured on Blood agar, MacConkey and nutrient agar. While urine samples were cultured on CLED agar. These culture Media were kept in incubator at 37 °C for 24 hours. Smear was prepared from individual purified colony on glass slide. Smear was heat fixed.

2.2 Gram staining
Gram staining was performed to isolate gram negative bacteria from gram-positive. Smear was flooded with Primary stain crystal violet for one minute. Excessive stain was removed with help of blotting paper. Then smear was washed with tap water. Then Gram iodine was poured on smear and allowed it to stand for one minute. After it, smear was flooded with decolorizing agent (95% ethanol) for 1 minute. At the end of staining a counter stain safranine was poured over the smear for 30-60 seconds. Slides were dried in air and then examined under the compound microscope. Gram positive bacteria retained colour of crystal violet while gram-negative bacteria appeared reddish as they retained the color of counter stain safranine.

2.3 Identification of bacteria
Bacteria were identified on the basis of morphological, colonial and biochemical characterizations. In order to study resistance pattern of gram-negative bacteria disc diffusion method was performed. For antimicrobial susceptibility test Muller Hinton media was used. A pure colony of bacteria was picked with the help of sterile loop and inoculated in saline solution. A sterile swab was used to pour bacterial sample on Muller Hinton media.

2.4 Antibiotic susceptibility test
Antibiotic discs of Amikacin, ampicillin, Gentamycin, ceftazidime and meropenem were placed over the media and were kept for 24 hours in incubator at 37 °C. A ruler was used to measure zone of inhibition. Then resistance patterns of different bacteria against number of antibiotics were observed.

3. Results and Discussion

3.1 Prevalence of clinical samples among gender
The infection rate was more prevalent in females as

compared to males. Majority of samples n=33(55%) were Female collected from females and n=27(45%) samples were obtained from males. Females are more prone to bacterial infections as compared to males due to anatomical, physiological, behavioral and hormonal imbalance. UTIs are more common in females as compared to males due to anatomical characteristics of urethra and its close proximity to anus. This shows that females are more prone to bacterial infections.
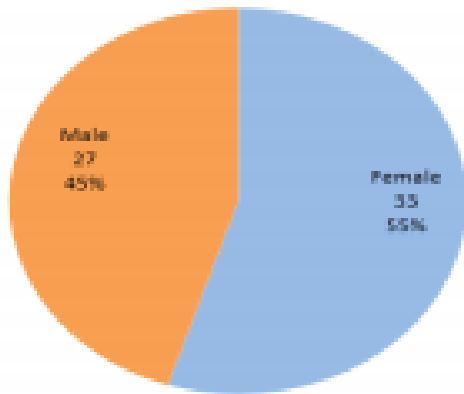

Figure 1: Prevalence of clinical samples among gender Prevalence of clinical samples.

The prevalence of different clinical samples is explained in Figure 2. Higher number of samples were of blood n=20(33%) as most people were suffering from bacteremia. Blood samples were followed by urine samples n=18(30%). Urine samples were collected from patients suffering from infections of urinary tract.
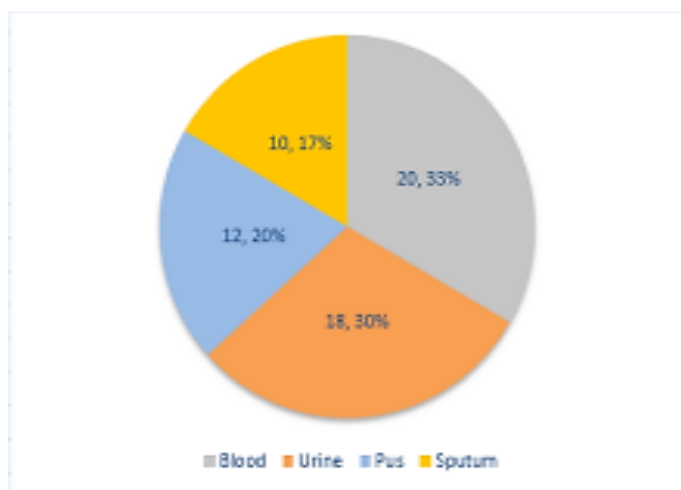

Figure 2: Prevalence of clinical samples.

Pus samples n=12(20%) were obtained from wounds. While minimum number of samples were of sputum n=10(17%).

3.2 Prevalence of bacterial isolates in clinical samples

Data in Figure 3 illustrates the prevalence of different bacterial isolates in clinical samples. *E. coli* was most prevalent (n=30) in clinical samples. As it is commensal of gastrointestinal tract and is present everywhere in environment so it is more prevalent in clinical samples. *Pseudomonas aeruginosa* was second most abundant (n=13) isolate in clinical samples followed by *Klebsiella* spp. (n=12) and *Proteus* spp. (n=5). These isolates were obtained from body fluids such as blood, urine, pus and sputum.
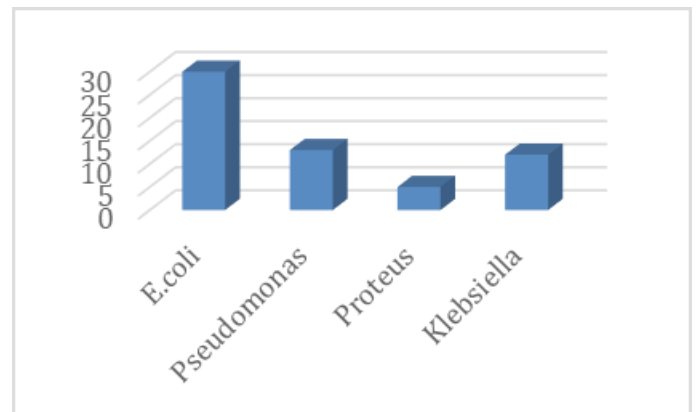

Figure 3: Prevalence of bacterial isolate in clinical samples.

The resistance patterns of different gram negative bacteria obtained from clinical samples were observed against most zcommonly used antibiotics. Total six antibiotics were used, which are most commonly used to treat bacterial infections. Different pathogens have shown different resistance patterns against these antibiotics Amoxicillin (AMX), Amikacin (AMK), Ciproflaxin (CIP), Ceftazidime (CAZ) Gentamycin (GEN) and Meropenem (MEM) were used.
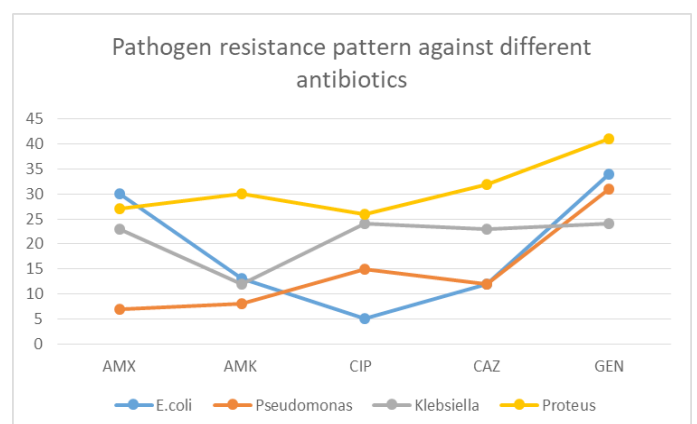

Figure 4: Pathogen resistance pattern agaisnt different antibiotics.

Due to abundance of multi-drug resistant microbes,

it has become difficult to treat the bacterial infections as very few antibiotics have left as option of chemotherapeutic agents (Cerceo *et al.*, 2016). In present study infection rate was most prevalent in females (55%) as compared to males (45%). The occurrence of UTIs varies according to different ages and genders, but its frequency of occurrence is higher in female as compared to male, which is due to multiple reasons for example anatomy of urethra, its occurrence near the anus, and different hormonal balance in female .UTIs are more prevalent in female especially in pregnancy duration (John *et al.*, 2016). Females are affected from UTIs in gestation period, and it leads to abortion, underdevelopment of infants and premature births. If it is treated at early stage it is beneficial for mother and fetus, but if it is not treated early it may have severe outcomes (Hannan *et al.*, 2012).

The Prevalence of UTIs also varies according to age which demonstrates that age is one risk factor for UTI. Older females are more affected from UTI as compared to young ones due to degeneration of urogenital tract and impairment of vagina after menopause. Due to these reasons the pH of vagina increases and number of vaginal *Lactobacillus* decrease which allow the gram-negative bacteria to settle down and increase in number and act as uropathogens, so UTI is more prevalent in older females (Nicolle, 2016).

Urine samples n=18(30%) were collected from patients suffering from infections of urinary tract. One of the most prevalent clinical complications is Urinary tract infections (UTIs), it includes the infections of kidneys, ureters, urinary bladder, and urethra (Haider *et al.*, 2010).

In present study n=12(20%) pus samples were obtained from wounds. Wounds are the reservoir of bacteria as they provide nutrition, temperature and humidity to them thus allow bacteria to divide rapidly and form the colonies at the infected site. Wounds are produced as a result of cuts on skin, operations cuts, accidents, some diseases such as Diabetes. The skin act as first line of defense in body but when wounds occur this is destroyed which allows bacteria to invade and cause infections. That's why wound infections are responsible for variety of infections, longer hospital stays, burden on economy and causalities all around the world. The prominent gram negative bacteria isolated from clinical samples are members of family

Enterobacteriaceae and *pseudomonas aeruginosa* (Sisay *et al.*, 2019).

Sputum samples n=10(17%) were collected from patients suffering from respiratory tract infections especially Pneumonia. One of the common hospital-acquired infections is Hospital-acquired Pneumonia (HAP) which results in longer stays in hospitals, enhanced encumbrance, and high causality rate globally. HAP is the second notorious hospital acquired infection in United States. HAP is caused by bacteria, so it becomes essential to study these bacteria which are causative agents of it. Gram-negative bacteria are prominent cause of pneumonia. HAP results in prolonged hospital stays, economic burden, and causalities.

E.coli was most prevalent isolate (50%) in clinical samples. *E. coli i*solates obtained from clinical samples were resistant against Amoxicillin, Ceftazidime, Gentamycin, Ciproflaxin and Meropenem. The resistance recorded in these isolates was much greater than the previous studies conducted by other researchers. The study has confirmed that antibiotic resistance is continuously increasing in isolates collected from patients suffering from gram-negative bacterial infections (Olorunmola *et al.*, 2013).

Pseudomonas aeruginosa was second most abundant isolate (22%) in clinical samples. *Pseudomonas aeruginosa* is widely distributed in all natural environments. This pathogen is responsible for urinary tract infections, blood born infections and severe form of pneumonia. This microbe can exist in two forms in environments either in free swimming form in water bodies or it forms the biofilms. But in special conditions it loses its mobility, comes to surface of water bodies and becomes non-motile (Khan *et al.*, 2010).

While *Klebsiella* shows maximum resistance towards Cefotaxime and Gentamycin. In a study conducted in India which showed that majority of *Klebsiella* is sensitive towards Amikacin. As has proven from this study that *Klebsiella* is 98% sensitive towards Amikacin. The reason of this susceptibility is that this medicine is not commonly used to treat the infections rather it is rarely used in case of infections (Sarathbabu *et al.*, 2012). A study which was conducted in Jordan has confirmed that *Klebsiella* is least resistant towards one antibiotic (Alsohaili *et al.*, 2015).

In present study *Proteus* (8%) was present in least numbers. *Proteus* showed maximum sensitivity towards Amikacin and this antibiotic is used to treat the infections. In another study conducted in Saudi Arabia has same results were obtained and its proven that majority of this pathogen is resistant towards Ampicillin. The results of present study have shown that this pathogen shows resistance towards Amoxicillin (Bahashwan and Shafey, 2013).

Conclusions and Recommendations

Multidrug resistant microbes are emerging rapidly in clinical environments and natural settings. Females are more infected with pathogenic bacteria than males. This is because of anatomical, hormonal, behavioral and physiological differences in two genders. Now a days bacteria are resistant against most commonly used antibiotics. Inappropriate and extensive use of antibiotics leads to emergence of multidrug resistance in microbes.

Novelty Statement

Monthly collection of resistant bacteria according to gender and clinical samples is of paramount importance. Identification of these strains help to design strategies to curtail the emergence and spread of resistance and to devise innovative therapeutic approaches against multidrug-resistant organisms.

Author's Contribution

Mehwish Saleem khan: Performed experiment, wrote paper.
Sumama Farooq: Edited the paper.

Conflict of interest
The authors have declared no conflict of interest.

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