Antimicrobial activity of broad spectrum Antibiotics against different clinical isolates

Javeria Ameer[1], Faiza Khan[1], Maqsood Ahmed Khan[1],Shazia Alam[1] ,Mehwish Murad Ali[1],Syed Adeel Ahmed Shah[1].

[1] Department of Pharmaceutics, Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan

ABSTRACT:

Concern over the development of antibiotic resistance is increasing among healthcare providers who offer their services globally. This study aims to detect the resistance pattern of some widespread microorganisms by calculating their zone of inhibition against some typically prescribed broad spectrum antibiotics. ***Escherichia coli*, *Staphylococcus aureus*** and ***Pseudomonas aeruginosa*** were among the prevalent clinical isolates that were gathered from a pathological laboratory in Karachi. Additionally, the pattern of their resistance to various broad spectrum antibiotics such as **Ciprofloxacin (5µg), Amoxicillin (25µg), Gentamycin (10µg), Cefotaxime (30µg), Ceftriaxone (30µg), and Clarithromycin (15µg)** was investigated by using disc diffusion method. The results revealed that all three selected strains were resistant to all the antibiotics in spite of the fact that zones of inhibition were observed in the antibiotics ciprofloxacin and gentamycin against pseudomonas aeruginosa strains.

Keywords: *Escherichia coli, Staphylococcus aureus, Pseudomonas*, *Staphylococcus aureus* clinical isolates.

INTRODUCTION:

Antimicrobial resistance is a hazard to global health and development and is responsible for 700,000 annual fatalities worldwide (Baekkeskov et al., 2020). If not treated quickly and efficiently, serious bacterial infections such bacteremia, sepsis, and pneumonia are associated with high death rates. Overuse of antibiotics during the past 60 years has led to the selection of resistant strains, raising the incidence of fatal infectious illnesses and placing a financial load on society. Although the existence of this issue is commonly acknowledged on a worldwide scale, many parts of the world lack a clear understanding of its scope. There hasn't been any published systemic analysis of antimicrobial resistance (AMR) in Pakistan up to this point. (Saeed et al., 2021) (Bilal et al., 2021).

The community, hospitals, and other healthcare facilities are all places where these illnesses can be contracted. Most of the time, medication must begin before the infection's causative organism is identified. Regarding the proper treatment of serious bacterial infections, the Infectious Diseases Society of America, the American Thoracic Society, the Society for Healthcare Epidemiology of America, and the Paul Ehrlich Society of Chemotherapy have all recently recommended the use of initial empirical therapy with broad-spectrum antimicrobials. Optimising the selection and duration of empirical antimicrobial therapy is one objective in reducing the emergence of antibiotic resistance. The type of care (outpatient, inpatient nonintensive care unit, or inpatient intensive care unit [ICU]), the common local pathogens and

their resistance patterns, and patient-specific risk factors for colonisation or infection with multidrug-resistant pathogens can all have an impact on the empirical regiment that is chosen. These factors are very important in choosing the best and most efficient treatment strategy. Systemic infections are frequently brought on by pathogenic bacteria, especially Gram-negative pathogens including *Escherichia coli, Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. (Morel et al., 2010). Bacterial resistance is the development of new defense mechanisms inside the bacteria that can thwart human defenses and antimicrobial drugs used to treat it (Beceiro et al., 2013).

The fifth most populous country in the world, Pakistan, has reported high levels of antibiotic resistance, adding to the strain on an already overburdened and underfunded healthcare system. Additionally, there have been reports of bacteria species with circulating mobile genetic components that can transfer multidrug resistance genes both within and across species. Furthermore, the discovery of extensively drug-resistant (XDR) *Salmonella typhi* in Hyderabad, Pakistan raises more questions about the intricate interactions between the environment and human activity that lead to the emergence of AMR. (Khan et al., 2011)

Broad spectrum—antibiotics, like amoxicillin, work against both gram positive and gram negative bacteria. (Pulingam et al., 2022). It is a significant factor in the rising rates of illness and death since the ratio of newly arising resistance to the discovery of new antibiotics is significantly greater than one another (Mossialos et al., 2010). Interestingly the compounds extracted from grampositive and gram-negative bacteria themselves are used to make antimicrobial medications. (Helander et al., 1997). Ciprofloxacin a fluoroquinolone antibiotic is used to treat a variety of illnesses, including chronic bacterial prostatitis, acute uncomplicated cystitis, urinary tract infections, and acute sinusitis (Masadeh et al., 2015). Amoxicillin used to treat infections of the tonsils, throat, larynx, pharynx, middle ear (otitis media),lungs (pneumonia), urinary tract, gonorrhea, typhoid fever, early Lyme disease, erythema migrans, sinusitis, gastritis & peptic ulcers, and meningitis condition. (Kaur and Nanda., 2011).

The National Action Plan of Pakistan for Antimicrobial Resistance, which was translated from the National Strategic Framework for Containment of Antimicrobial Resistance, was introduced in 2018 along with the national AMR surveillance system (PASS) that is in line with GLASS. In response to the Global Action Plan and reports of growing resistance in Pakistan, this was carried out. (Saeed et al., 2021)

Infections of the skin, soft tissues, and joints, as well as sexually transmitted diseases like gonorrhoea, community-acquired pneumonia, complicated urinary tract infections, and lower respiratory tract infections are all commonly treated with third-generation cephalosporins (Cefotaxime and ceftriaxone). (Lamb et al., 2002) (Wieland et al., 2012) (Hustig and Waddell., 2013).

Clarithromycin is a macrolide effective against pathogens responsible for respiratory tract infections and atypical pneumonia. It is a well-tolerated, acid-stable macrolide used against Helicobacter pylori (Logan et al., 1994).

For the selection of the most suitable empirical antibiotic therapy, ongoing surveillance data is needed. In order to create a worldwide action plan to stop AMR, the World Health Organization (WHO) also exhorts all nations to improve their AMR surveillance systems. In order to assess the efficacy of frequently used antibiotics and provide guidelines for the empirical treatment of all infectious diseases, such studies of antibiotic susceptibility at the regional and national levels should be carried out on a regular basis. (Saeed et al, 2021) Such kind of antimicrobial resistance studies is rare in Pakistan and continuously needed to evaluate the pattern of antimicrobial resistance.

The aim of this study is to check the effect of broad spectrum antibodies against common pathogens using disc diffusion method.

MATERIAL & METHOD:

Design of the study

Design of the study is experimental.

Test Organisms

clinical isolates of *Staphylococcus aureus*, *Esherichia coli*, *Pseudomonas aeruginosa* . These microorganisms were obtained from the laboratory stock of clinical microbiology laboratory of a private hospital Karachi Pakistan . They were maintained on agar slants at 4 ^{0}C in the refrigerator.

Antibiotics and Microbiological Media

The antimicrobial agents used were: amoxicillin (Oxoid Ltd);. ciprofloxacin (Oxoid Ltd);Gentamycin(Oxoid Ltd); Cefotaxime(OxoidLtd) ceftriaxone (Oxoid Ltd and clarithromycin (Oxoid Ltd) nutrient broth and nutrient Agar (Oxiod Ltd).nutrient agar(Oxoid Ltd) and MacCornkey agar Oxoid Ltd).

Testing for antimicrobial susceptibility

On Nutrient Agar (NA) and MacConkey agar drug susceptibility testing (DST) was carried out using the disc diffusion method. Three colonies were emulsified in sterile saline and the suspension's turbidity was adjusted to the 0.5 McFarland standard.

On the inoculated agar surface, 6 commercially manufactured, fixed concentration paper antibiotic discs are put. Before determining the results, the plates were incubated for 16-24 hours at 35°C. The growth inhibition zones around each antibiotic disc were measured to the

closest millimeter using Vernier caliper. The zone's diameter is proportional to the isolate's susceptibility and the rate of drug diffusion through the agar media. The zone diameters of each drug are interpreted using the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards 2022).

RESULTS:

In this study the resistant pattern of clinical isolates of *Escherichia coli, Staphylococcus aureus*, and *Pseudomonas aeruginosa* was determined using antibiotics Ciprofloxacin (5µg), Amoxicillin (25µg), Gentamycin (10µg), Cefotaxime (30µg), Ceftriaxone (30µg), and Clarithromycin (15µg). The results revealed that *Pseudomonas aeruginosa* showed resistance against all inspected antibiotics except ciprofloxacin and gentamycin and the zones of inhibition. In the case of *Staphylococcus aureus* zone of inhibition was observed only with Gentamycin. On the other hand, bacterial strain *E. coli* showed resistance against all antibiotics, and no zone of inhibition was observed as depicted in Table 1.

Table 1: Comparison among the zones of inhibition (mm) of Ciprofloxacin (5µg), Amoxicillin (25µg), Gentamycin (10µg), Cefotaxime (30µg), Ceftriaxone (30µg), and Clarithromycin (15µg) against different bacterial strains

ANTIBIOTICS	Ciprofloxacin (5µg)	Amoxicillin (25µg)	Gentamycin (10µg)	Cefotaxime (30µg)	Ceftriaxone (30µg)	Clarithromycin (15µg)
Pseudomonas aeruginosa	S (38 mm)	R	S (100 mm)	R	R	R
E- Coli	R	R	R	R	R	R
Staphylococcus aureus	R	R	S (28 mm)	R	R	R

Where, S = Sensitive, R = Resistant

DISCUSSION:

The resistance demonstrated by *Pseudomonas aeruginosa*and *E. coli* to multiple antibiotics raises concerns about multi-drug resistance (MDR). MDR occurs when bacteria develop resistance to different classes of antibiotics, severely limiting treatment options. The high level of resistance observed in these clinical isolates underscores the urgent need for novel therapeutic approaches and effective antimicrobial stewardship to combat MDR.

The results highlight the importance of selecting appropriate antibiotics based on sensitivity testing. Ciprofloxacin and gentamycin demonstrated effectiveness against *Pseudomonas*

aeruginosa, indicating their potential utility in treating infections caused by these strains. Conversely, the resistance of *E. coli* to all tested antibiotics, including ciprofloxacin, emphasizes the challenge in managing infections caused by this pathogen and highlights the need for alternative treatment strategies.

In 2011, Khan used erythromycin and clarithromycin to examine the resistance patterns of fifty (50) clinical isolates of *Escherichia coli, Staphylococcus aureus, Klebsiella,* and *Proteus*. The findings showed that erythromycin resistance was present in 23.54% of clinical isolates of *Escherichia coli*, 27.78% of *Staphylococcus aureus*, 41.67% of *Klebsiella*, and 66.67% of *Proteus*. Clarithromycin resistance was found in 35.30% of clinical isolates of *Escherichia coli*, 22.23% of *Staphylococcus aureus*, 58.34% of *Klebsiella*, and 66.67% of *Proteus*. Gentamycin displayed inhibitory activity against *Staphylococcus aureus*, suggesting it can still be considered as a viable treatment option for infections caused by this bacterium. However, further investigation into the prevalence and mechanisms of resistance to Gentamycin among clinical isolates is necessary to ensure its continued effectiveness.

The high resistance exhibited by *Pseudomonas aeruginosa* to the tested antibiotics emphasizes the urgency in understanding the underlying mechanisms of resistance in these organisms. Research efforts should be directed towards developing new drugs or combination therapies to combat infections caused by these challenging pathogens.

The study highlights the importance of antimicrobial stewardship programs in healthcare settings. These programs promote the appropriate use of antibiotics, reduce the development of resistance, and preserve the effectiveness of existing antibiotics. Implementing such programs can help mitigate the spread of antibiotic-resistant bacteria and improve patient outcomes.

Masadeh et al in 2015 looked into the potential attenuating effects of PDE inhibitors on the antibacterial activity of ciprofloxacin against a variety of reference bacteria, including E coli, *Staphylococcus aureus, Pseudomonas aeruginosa, Staphylococcus epidermidis, A baumannii, Proteus mirabilis*, and *K pneumoniae*. The Clinical and Laboratory Standards Institute's recommended inhibition zones were regarded as an accurate representation of bacterial susceptibility to the chemicals. With the exception of *A baumannii* and *K pneumonia*, which exhibited a zone of inhibition in the intermediate and resistant ranges, ciprofloxacin had considerable antibacterial activity against all of the reference bacteria that were examined.

Measurements of zones of inhibition were used to assess the resistance of clinical isolates. According to the findings of the current investigation, ceftriaxone (30 g) was highly effective against *E. coli* (90%). *S. aureus* was also susceptible to the medication, but not to the same degree as *E. coli*. The least susceptible organism, *K. pneumoniae*, a gram-negative facultative bacterium from the Enterobacteriaceae family, only showed a 65% sensitivity. According to reports, genetic modifications are causing *Klebsiella* organisms to become resistant to a number of antibiotics. The emergence of extended-spectrum beta-lactamase (ESBL) *Klebsiella pneumoniae* is posing a danger to doctors' ability to manage community acquired illnesses brought on by *Klebsiella*. (Bushra et al., 2016)

In a study on the prevalence of antibiotic resistance among gram-negative isolates in an adult intensive care unit in a tertiary care facility, *Klebsiella* and *E. coli*, among other pathogens, were shown to be less susceptible to different antibiotics. (Al-Johani et al., 2010)

The incidence of MRSA and its pattern of antibiotic susceptibility were described by Rajaduraipandi et al in 2006. Penicillin resistance was present in 99.6% of clinical MRSA isolates, ampicillin resistance was present in 93.6%, and gentamicin, co-trimoxazole, cephalexin, erythromycin, and cephotaxime resistance was present in 63.2%. Penicillin resistance was present in all MRSA strains (100%) in carrier screening samples, whereas ampicillin and co-trimoxazole resistance rates were correspondingly 71.8% and 35.9%. 23% of carrier MRSA isolates and 63.6% of clinical MRSA isolates were found to be multidrug resistant. Vancomycin was, however, susceptible to all strains of clinical and carrier individuals.

In a study on the prevalence of antibiotic resistance among gram-negative isolates in an adult intensive care unit in a tertiary care facility, *Klebsiella* and *E. coli*, among other pathogens, were shown to be less susceptible to different antibiotics. (Al-Johani et al., 2010)

It has been discovered that the practise of prescribing a certain class of antibiotics to specific organisms plays a crucial part in the development of resistance against that antibiotic. (Costelloe et al., 2010).

Conclusion:

This study sheds light on the antimicrobial activity of broad-spectrum antibiotics against clinical isolates of *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The observed resistance patterns underscore the need for a cautious approach to antibiotic use and the urgent development of new therapeutic strategies to combat multi-drug resistant bacterial infections. Antimicrobial stewardship programs play a crucial role in promoting responsible antibiotic use and preserving the efficacy of existing treatments. Future research should focus on exploring alternative treatment options and understanding the molecular mechanisms behind resistance to better address this growing public health concern.

REFERENCES:

Abbas, A., Hasnain, A., Malik, S., Kausar, H., Siddiqui, M.F., Mehboob, R. and Chaudhry, S., 2022. Comparison of the Antibacterial Activities of Different Antibiotics Against Clinical Isolates: Antibiotic Resistance Patterns. *Pakistan BioMedical Journal*, pp.49-53.

Al Johani SM, Akhter J, Balkhy H, El-Saed A, Younan M and Memish Z (2010). Prevalence of antimicrobial resistance among gram-negative isolates in an adult intensive care unit at a tertiary care center.

Baekkeskov, E., Rubin, O., Munkholm, L. and Zaman, W., 2020. Antimicrobial resistance as a global health crisis. In *Oxford Research Encyclopedia of Politics*.

Beceiro, A., Tomás, M. and Bou, G., 2013. Antimicrobial resistance and virulence: a successful or deleterious association in the bacterial world?. *Clinical microbiology reviews*, *26*(2), pp.185-230.

Bilal, H., Khan, M.N., Rehman, T., Hameed, M.F. and Yang, X., 2021. Antibiotic resistance in Pakistan: a systematic review of past decade. *BMC infectious diseases*, *21*(1), pp.1-19.

Bushra, R., Sial, A.A., Rizvi, M., Shafiq, Y., Aslam, N. and Bano, N., 2016. Sensitivity pattern of ceftriaxone against different clinical isolates. *Pakistan Journal of Pharmaceutical Sciences*, *29*(1).

Costelloe C, Metcalfe C, Lovering A, Mant D and Hay AD (2010). Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. BMJ., 340: c2096.

Gurung, R.R., Maharjan, P. and Chhetri, G.G., 2020. Antibiotic resistance pattern of *Staphylococcus aureus* with reference to MRSA isolates from pediatric patients. *Future science OA*, *6*(4), p.FSO464.

Helander, I., von Wright, A. and Mattila-Sandholm, T.M., 1997. Potential of lactic acid bacteria and novel antimicrobials against Gram-negative bacteria. *Trends in Food Science & Technology*, *8*(5), pp.146-150.

Hustig, A., Bell, C. and Waddell, R., 2013. An audit of pharyngeal gonorrhoea treatment in a public sexual health clinic in Adelaide, South Australia. *International journal of STD & AIDS*, *24*(5), pp.399-400.

Kaur, S.P., Rao, R. and Nanda, S.A.N.J.U., 2011. Amoxicillin: a broad spectrum antibiotic. *Int J Pharm Pharm Sci*, *3*(3), pp.30-7.

Khan, N.W., Hassan, F., Naqvi, B.S. and Hasan, S.M.F., 2011. Antimicrobial activity of Erythromycin and Clarithromycin against clinical isolates of *Escherichia coli, Staphylococcus aureus, Klebsiella and Proteus* by disc diffusion method. *Pak. J. Pharm. Sci*, *24*(1), pp.25-29.

Kibret, M. and Abera, B., 2011. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. *African health sciences*, *11*, pp.40-45.

Lamb, H.M., Ormrod, D., Scott, L.J. and Figgitt, D.P., 2002. Ceftriaxone: an update of its use in the management of community-acquired and nosocomial infections. *Drugs*, *62*(7), pp.1041-1089.

Logan, R.P., Gummett, P.A., Schaufelberger, H.D., Greaves, R.R., Mendelson, G.M., Walker, M.M., Thomas, P.H., Baron, J.H. and Misiewicz, J.J., 1994. Eradication of Helicobacter pylori with clarithromycin and omeprazole. *Gut*, *35*(3), pp.323-326.

Masadeh, M.M., Alzoubi, K.H., Khabour, O.F. and Al-Azzam, S.I., 2015. Ciprofloxacin-induced antibacterial activity is attenuated by phosphodiesterase inhibitors. *Current Therapeutic Research*, *77*, pp.14-17.

Morel, J., Casoetto, J., Jospé, R., Aubert, G., Terrana, R., Dumont, A., Molliex, S. and Auboyer, C., 2010. De-escalation as part of a global strategy of empiric antibiotherapy management. A retrospective study in a medico-surgical intensive care unit. *Critical care*, *14*(6), pp.1-7.

Mossialos, E., Morel, C.M., Edwards, S., Berenson, J., Gemmill-Toyama, M., Brogan, D. and World Health Organization, 2010. *Policies and incentives for promoting innovation in antibiotic research*. World Health Organization. Regional Office for Europe.

Pulingam, T., Parumasivam, T., Gazzali, A.M., Sulaiman, A.M., Chee, J.Y., Lakshmanan, M., Chin, C.F. and Sudesh, K., 2022. Antimicrobial resistance: Prevalence, economic burden, mechanisms of resistance and strategies to overcome. *European Journal of Pharmaceutical Sciences*, *170*, p.106103.

Pullens, B. and van Benthem, P.P., 2011. Intratympanic gentamicin for Meniere's disease or syndrome. *Cochrane Database of Systematic Reviews*, (3).

Rajaduraipandi, K., Mani, K.R., Panneerselvam, K., Mani, M., Bhaskar, M. and Manikandan, P., 2006. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: A multicentre study. *Indian journal of medical microbiology*, *24*(1), pp.34-38.

Saeed, D.K., Farooqi, J., Shakoor, S. and Hasan, R., 2021. Antimicrobial resistance among GLASS priority pathogens from Pakistan: 2006–2018. *BMC infectious diseases*, *21*, pp.1-16.

Talebi Bezmin Abadi, A., Rizvanov, A.A., Haertlé, T. and Blatt, N.L., 2019. World Health Organization report: current crisis of antibiotic resistance. *BioNanoScience*, *9*, pp.778-788.

Wieland, B.W., Marcantoni, J.R., Bommarito, K.M., Warren, D.K. and Marschall, J., 2012. A retrospective comparison of ceftriaxone versus oxacillin for osteoarticular infections due to methicillin-susceptible Staphylococcus aureus. *Clinical infectious diseases*, *54*(5), pp.585-590.