Bulletin of Biological and Allied Sciences Research
***ISSN:* 2521-0092**
www.bbasr.org
DOI: https://doi.org/10.54112/bbasr.v2023i1.48
*Bull. Biol. All. Sci. Res., Volume, **8**: 48*

Original Research Article

ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF VARIOUS BACTERIA ISOLATED FROM RESPIRATORY TRACT INFECTION

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(Received, 17th January 2023, Revised 2nd October 2023, Published 4th October 2023)

Abstract *The most common and wide types of infections are Respiratory tract infections (RTIs), known for high morbidity and mortality in medicine. This study was conducted to determine the microbial pathogens responsible for respiratory tract infections and their antimicrobial susceptibility pattern. Total 130 sputum and 70 swabs were collected and processed according to standard laboratory procedures Absyn University Peshawar, Pakistan microbiology laboratory. The samples were processed to screen pathogenic bacteria causing respiratory tract infections. Of all samples, the most prevalent bacteria were enterococcus (29.2%) followed S. aureus (27%), S. pneumoniae (14.0%), M. catarhalis (8.4%), K. pneumoniae (7.3%), S. pyogenes (7.9%), and H. Influenza (6.2%). Antibiotics susceptibility profile was done to determine resistance level in isolated species against current antibiotics. It concluded that different bacterial species were responsible for URT and LRT infections and were detected as multi-drug resistance. Further molecular research is needed to identify resistance genes among these species.*

*[Citation: Abdullah, Amin, F.U., Neelma, H., Bashir, K., Khan, A.A., Bibi, H., Isrhad, M., Khan, S., Nawaz, K., Ullah, Z. (2023). Antimicrobial Susceptibility profile Of Various Bacteria Isolated from Respiratory Tract Infection. Bull. Biol. All. Sci. Res. **8**: 48. doi:* https://doi.org/10.54112/bbasr.v2023i1.48*]*
Keywords: Antimicrobial, *Multi Drug resistance, respiratory tract infection, antibiotics*

Introduction

The utmost communal and wide types of infections are included in Respiratory tract infections (RTIs), known for high morbidity and mortality in medicine (Malosh et al., 2018). It is widely accepted that the respiratory tract is one of the most common sites of infection as it interacts directly with the physical environment and is exposed to airborne microbes such as fungi, bacterial species, and viral parasites. Respiratory tract infection (RTIs) includes upper and lower respiratory tract infection and are considered the most significant infectious disease globally facing both developed and developing countries (Kumari et al., 2007). RTI mainly occurs in upper respiratory tract infections (URTI), presents a higher incidence in children and adults, and has an important economic influence related to lost output in the workplace and physicians' higher antibiotic prescriptions rate (Atia et al., 2018). The most commonly found infections in upper respiratory tract infections (URTIs) are common cold, laryngitis, pharyngitis, otitis media rhinitis, and tonsillitis (Assane et al., 2018). The most common bacterial species causing infection in the upper respiratory tract are *Haemophilusinfluenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Moraxella catarrhalis* and *Streptococcus pyogenes* (Watson et al., 2006).

Lower respiratory tract infections (LRTIs) are among the most common causes of morbidity and mortality worldwide (El-Mahmood et al., 2010; Mishra et al., 2012). In LRTIs the causative agents are mostly gram-negative bacterial pathogens. Numerous studies have been conducted on the LRTIs and reported that a large proportion of gram-negative bacteria are responsible for the infection in current years, especially in hospital-acquired infections and in patients from the community (Dahiya and Purkayastha, 2012; Karaiskos and Giamarellou, 2014). It generally happens when pathogenic microbes reach the parenchyma pulmonary airway by crossing the upper respiratory tract's mechanical and further nonspecific barriers. Infection may occur due to inhaling infectious aerosols, aspiration of oral or gastric contents, or heterogeneous spread (Mahon and Lehman, 2022; Yusuf and Hamid, 2017). The bacterial specimens and symptoms of respiratory diseases diverge with age, gender, season, the type of population at risk, and other factors (Mishra et al.,

2012; Watson et al., 2006). LRTI's can be designated as infections showing symptoms such as fever, weakness, shortness of breath, coughing fatigue or discomfort, usually for a period ranging from 1-3 weeks. LRTIs include bronchitis and pneumonia commonly (Shrestha et al., 2013). Common intracellular parasites includes virus, fungi and pathogenic bacteria are involved in LRTIs. The most common bacteria are *streptococcus pneumoniae*, *K. pneumonia*, and *enterococcus species* and *H. influenza* (Uzoamaka et al., 2017).

URTIs are also due to the excessive use of antibiotics. Almost 60% of all antibiotics used are for respiratory infections, mainly acute bacterial sinusitis for adults and acute bacterial otitis for children (Jong et al., 2016; Weinstein and Lewis, 2020). The resistance of bacterial species to antibiotics effectiveness is increasing daily and is a serious concern for the globe. The developed countries are going to search for alternative treatments to treat multidrug-resistant microbes, the condition in poor countries may be untreatable (Ozyılmaz et al., 2005). Observing the resistance patterns of microbial pathogens is required to guide the clinician when managing cases requiring antibiotic therapy and monitor the tendency of these types of illness. Bacterial species are well-known to cause prime or superinfection; in most cases, they require targeting (Keith et al., 2010). The community-acquired infection is generally treated with empirical drugs of choice. Antibiotics are considered harmless, this idea leads to the emergence and speedy increase of resistance strains to antimicrobial and has problematic the selection practice of antimicrobial compounds (Uzoamaka et al., 2017). Multidrug-resistant (MDR) bacteria resist currently used antibiotics (Dahiya and Purkayastha, 2012). Bacterial resistance affects the entire globe in managing infectious diseases that result in high morbidity and mortality rates in the community (Organization, 2014).

Materials and Methods

Sample collection

Samples were collected from patients of different hospitals located in Peshawar. The samples include sputum as well as throat swabs. Sterile phlegm containers were used for sputum collection, and a sterile swab for throat samples. The samples were transported to the microbiology laboratory of Abasyn University Peshawar. The samples were inoculated on MacConkey agar (MA), blood agar (BA), and chocolate agar (CHA) media. After inoculation, plates were incubated at 37c for 24 hours.

Bacterial identification

All bacterial isolates were identified using microscopic, morphological, and biochemical techniques.

Morphology identification

After incubation positive agar plates were observed for colony morphology according to CLSI guidelines to give an initial assumption about the kind of bacteria that grow on agar plates (CLSI 2019). The bacteria were preliminary identified based on their colonial morphology, presence or absence of hemolysis on blood agar, fermenter or non-fermenter.

Microscopically Identification:

Gram's staining was done to confirm whether Gram-positive or Gram-negative cocci or bacilli.

Gram's Staining Technique

Thin, uniform smears of specimens were made on glass slides and were allowed to be air dried. Smears were fixed by heat and were allowed to cool. Crystal violet was used to flood on the slides and was allowed to stand for 1 minute, after that water was used to wash the slides. Gram's Iodine was allowed to flood on the slides for 1 minute and was cleaned with water. These slides were depolarized with Iodine acetone for 1 minute or differentiator for 10 seconds and were rinsed with water. Counter stain was allowed to flood on the slides for 30 – 60 seconds. These slides were properly rinsed with water, gently blotted, and allowed to be dried. Using oil immersion microscopy, slides were examined. Gram positive organisms gave blue to purple color in microscope. Gram negative organisms gave pink to red color in the microscope. Slides were also examined for bacterial morphology either bacilli, single cell, strept form, cocci, chain form, comma-shaped, letter-shaped, diplococcal etc. (Cobos-Trigueros et al., 2017).

Biochemical Identification:

Biochemical analyses were done for the confirmation of bacterial species. In chemical testing, some experiments showed on-the-spot result whereas others had to be incubated for a time. The following tests identify and characterize bacterial species (Hudzicki, 2009). Various essential tests identified the bacterial species include bile solubility and optochin sensitivity test for *S.pneumoniae*, bacitracin sensitivity test for *S. pyogenes*, and appropriate biochemical tests including TSI (Triple Sugar Iron), Citrate, MIU (Motility Indole Urea) media, for Enterobacteriaceae like *Klebsiella pneumoniae*. Pure cultures of Moraxella yielding oxidase, catalase test, no growth on nutrient agar at room temperature, failure to ferment glucose, lactose, mannitol and sucrose were considered significant.

Oxidase Test

Oxidase test was first performed for all gram negative organisms according to the manufacturer's procedure. The families of *Pseudomonadaceae*and *Enterobacteriaceae*are differentiated by oxidase test. Family *Pseudomonadaceae* is oxidase positive while *Enterobacteriacea*is oxidase negative. The bacteria produce cytochrome C oxidase was identified by using oxidase test, an enzyme used in the electron transport chain in bacteria. Tetra methyl-p-phenylenediamine is a reagent that is used in this test. If bacteria contain the enzyme purple color end product is formed. If not present, there is no colorful end product. A few drops of reagent were poured on the sterile paper and mixed with colony of test bacterial isolates, and a smear was formed with the

help of sterile stick.If the organism is oxidase positive, the phenylenediamine in the reagent will be reacted and produce deep purple color (Cobos-Trigueros et al., 2017).

Indole production Test

The experimental bacterial isolates were cultured on tryptophan containing media plates. The production of Indole was noticed when Kovac's or Ehrilch reagent containing 4 (p)-dimethylaminobenzaldehydereacted with indole and gave a red coloured complex. Kovac's reagent is suggested instead of Ehrilch's reagent for detecting indole from enterobacteria.

Catalase Test

The catalase test was used primarily to differentiate between the genus *Staphylococcus* and the genus *Streptococcus*. The genus Staphylococcus gave positive catalase reaction, whereas genus *Streptococcus* gave a negative catalase reaction. Catalase enzyme is present in aerobic and facultative anaerobic bacteria containing cytochrome except *Streptococcus* species. The test organism was exposed to hydrogen peroxide and oxygen production was observed. This test was especially important for gram positive bacteria.

1. Immediate bubbling - Positive reaction
2. No bubbling – negative reaction (Yusuf and Hamid, 2017).

DNase Test

Tryptophan in DNase Test Agar medium provide nutrients for bacterial growth. Sodium chloride maintain the osmotic balance. For the detection of deoxyribonucleic (DNase) Deoxyribonucleic acid in the agar plate was used that depolymerized DNA. After incubating the test strain in medium the plate was flooded with hydrochloric acid. The medium became opaque due to the precipitation of the polymerized DNA by HCL. A clear zone was produced around the area when organism degraded DNA (MacFaddin, 2000).

Coagulase Test

For the differentiation of *S. aureus* from *Staphylococci* coagulase test was used. Coagulase enzyme was produced by *S. aureus* which caused the plasma to clot.*S. aureus* produced the enzyme coagulase and caused citrated plasma to clot. There are two types of coagulase enzymes, bound and free coagulase. Bound coagulase was detected by slide coagulase test which absorbed fibrinogen from the plasma. In contrast, free coagulase was detected by tube coagulase test and it caused plasma to clot by converting fibrinogen to fibrin clot. The *S. aureus* causing clumping and resulting in the agglutination of cells. By tube coagulase test both free and bound coagulase can be detected (MacFaddin, 2000).

Antibiotics susceptibility profile

Kirby-Bauer Disk Diffusion Method

The Kirby-Bauer disk diffusion test is the most common and important antibiotic resistance/susceptibility testing method. For treating a patient, Kirby-Bauer disk diffusion testing results help physicians choose the correct antibiotics to be prescribed. This method used a Small filter disk with a known concentration of antibiotics. The test microorganisms were cultured on Muller- Hinton agar plates. The disks were placed on these inoculated Muller- Hinton agar plates. The antibiotics diffused from the disks into the surrounding agar plates during incubation. In case of susceptibility to the antibiotic, a clear zone was produced and test organism was not grown around the disk. The depth of the agar, the sensitivity of microbe to the antibiotic and the rate of diffusion of the antibiotic through the agar are the main factors responsible for the size of this zone. No zone of inhibition or a relatively small zone was given by microorganisms resistant to that antibiotic (Hudzicki, 2009).

Table 1: antibiotics used during the research work.

S. no	Antibiotics
1	Co-amoxyclav
2	Penicillin
3	Cefuroxime
4	Levofloxacin
5	Erythromycin
6	Gentamycin
7	Ciprofloxacin
8	Tetracycline
9	Ampicillin
10	Cefotaxime

Inoculum Standardization

Careful visual examination of agar plates having the test and control strains was done before inoculum preparation. A fresh sub – culture was prepared if cultures appeared mixed. A loop or sterile swab was touched to the top of at least 4 to 5 well isolated colonies. This was transferred to the Saline containing tube. To prevent clumping of the cells the inoculum was emulsified carefully inside the tube. It was ensured to pick sufficient bacterial numbers were when cells were picked from more than one colony.

Adjustment of Inoculum Standard to a 0.5 McFarland

The turbidity of inoculum was compared to that of the 0.5 McFarland standard using a paper with black lines or nephelometer. Turbidity of inoculum was adjusted in such a way as to match the turbidity of standard. 0.5 McFarland was approximately equal to 108 CFU/ml. For the accurate assay, a standardized inoculum was essential.

Inoculation of Muller- Hinton agar plates

Muller- Hinton agar plates were visually examined before use to ensure that plates have no contamination, approximately 4mm pouring depth, without excessively wet, dry, or cracks. Within 15 minutes of preparing the adjusted inoculum, a sterile cottons swabs dipped into the inoculum. To remove excess inoculum from the swab, the swab was rotated multiple times and pressed firmly above the fluid level

on the inside wall of the tube. The swab was streaked over the whole surface of the Muller- Hinton agar plates. Streaking motion was repeated and the plate was repeated 60º.The inoculation was completed by running the swab around the agar rim. Before applying the antimicrobial disks on the plates excess moisture was absorbed. For excess moisture to be absorbed, the plate lid was left open for 3 – 5 minutes.

Antimicrobial Disk Dispensing

The disks were dispensed on the agar surface using a disk dispenser or sterile forceps. After contact with the agar the location of the disk was not changed. To make a strong contact of the disks with the surface of agar the tops of the disks were pressed by a forceps.

Inoculum Purity Verifying

A nutrient agar plate was used by streaking the inoculum on the plate to check the purity of the inoculum. To collect inoculum from the tube and to be plated to a nutrient agar plate a sterile 10 µl loop was used. Plates were inverted and incubated for 16 to 18 hours at 37 °C.

Reading

Visually the purity of the control plate was checked. In case of mixed growth sub culturing was done to get pure culture. A confluent lawn of the growth was checked. Around some disks single colonies of resistant organisms were observed. When the inoculum was too light and the single colonies did not appear across the plate, the sample was tested again. Physical examination of the zone was done. Inhibition zones diameter was measured.

Interpretation of results

CLSI guideline 2019 was used to detect either resistant or sensitive test strain. For interpretation of the inhibition zones confluent lawn of growth and regression lines of a large population of isolates was used.

Reporting

Results were reported as either Susceptible (S), Intermediate (I), or Resistant (R) following interpretation according to the CLSI guidelines *(CLSI, 2019*). At last the percentage of sensitivity and resistant pattern of different antibiotics against different pathogens was calculated.

Results

The study designed to examine the bacterial species in respiratory tract infection followed by their identification and checked the antibiotic susceptibility profile of isolated bacteria. In the present study total 200 sputum and throat swabs were collected from different hospitals in Peshawar city. Specimens were investigated for the presence of pathogenic bacteria. Results revealed that all specimens showed bacterial growth on media plates.

Table: 2 Frequency distribution of samples from various hospitals.

S.NO	Location	Sputum samples	Throat swab	Percentage (%)
1	LRH	40	10	25
2	Alkhidmat Hospital	30	30	30
3	KTH	60	30	45

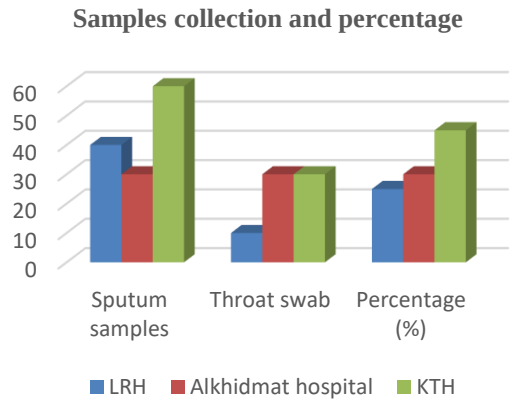

Figure: 1 Frequency distribution of samples from various hospitals.

In this study total 130 sputum samples for Lower respiratory tract and 70 throat swabs for upper respiratory tract infections were analyzed for presence of bacterial pathogens.

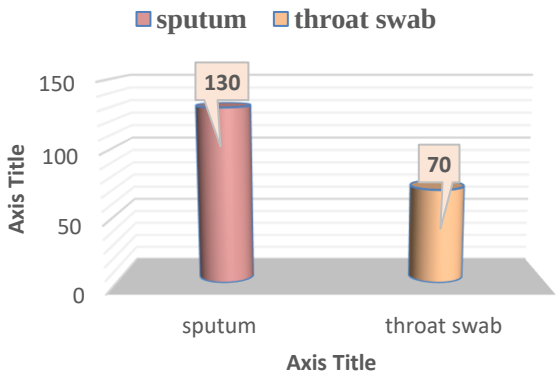

Figure: 2 frequency distributions of sputum and throat swabs.

Bacterial isolates were then processed for identification. Isolated bacteria were identified by morphologically, microscopically and biochemical testing. The bacteria isolates in the URTIs swabs and LRTIs sputum were: *enterococcus* (29.3%), *S. aureus* (27.4%), *S. pneumoniae* (14.0%), *M. catarhalis (8.4%). K. pneumoniae* (7.3%), *S. pyogenes* (7.9%), *H. Influenza (6.1%).*

Table: 3 Frequency distribution of bacteria from URTIs and LRTIs

Bacterial isolates	Frequency	Percent	Cumulative Percent
Enterococcus	52	29.2	29.2
H. influenza	11	6.2	35.4
K. pneumonia	13	7.3	42.7
M. catarhalis	15	8.4	51.1
S. aureus	48	27.0	78.1
S. pneumonia	25	14.0	92.1
S. pyogenes	14	7.9	100.0
Total	178	100.0	

During current study different antibiotics were checked against the isolated bacterial species. The bacterial strains were evaluated as resistant and sensitive by measuring the inhibitory zones around antibiotics disks. All the bacterial isolates showed higher level of resistance against ampicillin and penicillin.

Table: 4 Antibiotic sensitivity pattern of isolated bacterial

Antibiotics	*Streptococcus pneumonia*		*Klebseila Pneumoniae*		*Streptococcus pyogenes*		*Moraxella catarhalis*		*Hemophilus influenza*		*Enterococcus Spp*		*Staph aureus*	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
Ampicillin	99	1%	100	0	100	0	100	0	100	0	99	1	100	0
Penicillin	98	2	—	---	100	0	---	---	---	---	97	3	98	2
Co-amoxyclav	85	15	98	2	81	19	98	2	98	2	78	22	78	22
Cefotaxime	78	22	85	15	82	18	86	14	88	12	79	21	76	24
Cefuroxime,	82	18	90	10	87	13	89	11	90	10	86	14	80	20
Levofloxacin	25	75	55	45	40	60	55	45	15	85	15	85	16	84
Erythromycin	74	26	---	---	88	12	---	---	---	---	75	25	69	31
Gentamycin	18	82	53	47	16	84	60	40	20	80	20	80	15	85
Ciprofloxacin	30	70	65	35	45	55	67	33	45	55	20	80	18	82
Tetracycline	56	44	82	18	54	46	85	15	87	13	54	46	56	44

Antibiotics sensitivity pattern of *S. pnumoniae*: *P. pnumoniae*showed 99% resistance and 1% sensitive to Ampicillin followed by Penicillin against 98% resistance and 2% sensitive, 85 resistance and 15% sensitive to Co. amoxiclave, 82% resistance and 18% sensitive to Cefuroxime, 78% resistant and 22% sensitive to Cefotixime, 74% resistant and 26% sensitive to Erythromycin, 56% resistant and 44% sensitive to Tetracycline, 30% resistant and 70% sensitive to Ciprofloxacin, 25% resistant and 75% sensitive to Levofloxacin, 18% resistance and 82% sensitive to Gentamycin.

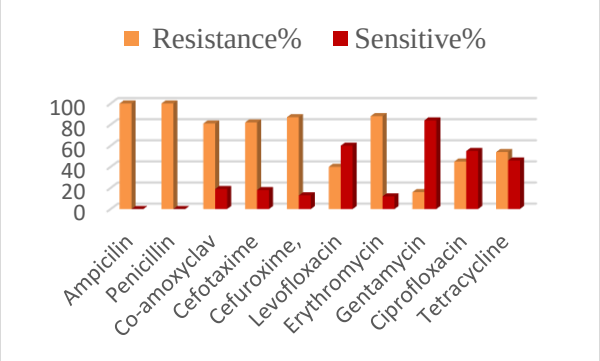

Figure: 3 Antibiotics sensitivity pattern of *S. pnumoniae*

Antibiotics sensitivity pattern of *K. pnumoniae*: *K. pnumoniae*was observed 100% resistance and 0% sensitive to Ampicillin followed by Co.amoxiclave against 98% resistance and 2% sensitive, 85 resistance and 15% sensitive to Cefoxatime, 90% resistance and 10% sensitive to Cefuroxime, 85% resistant and 15% sensitive to Cefotixime, 74% resistant and 26% sensitive to Erythromycin, 82% resistant and 18% sensitive to Tetracycline, 65% resistant and 35% sensitive to Ciprofloxacin, 55% resistant and 45% sensitive to Levofloxacin, 53% resistance and 47% sensitive to Gentamycin.

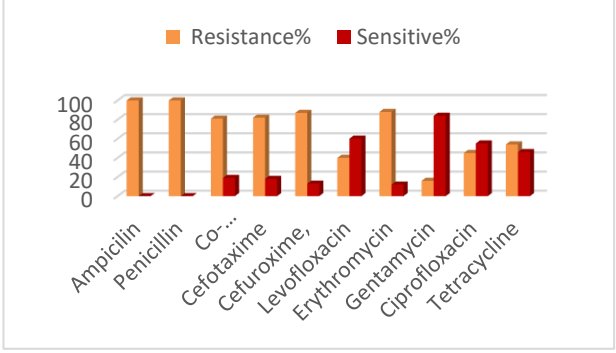

Figure: 4 Antibiotics sensitivity pattern of *K. pnumoniae*

Antibiotics sensitivity pattern of *S. pyogenes:* Results showed that***S. pyogenes***was observed 100% resistance and 0% sensitive to Ampicillin followed by Penicillin against 100% resistance and 0% sensitive, 88% resistance and 12% sensitive to Erythromycin, 87% resistance and 13% sensitive to Cefuroxime, 82% resistant and 18% sensitive to Cefotixime, 81% resistant and 19% sensitive to Co-amoxiclave, 54% resistant and 46% sensitive to Tetracycline, 45% resistant and 55% sensitive to Ciprofloxacin, 40% resistant and 60% sensitive to Levofloxacin, 53% resistance and 47% sensitive to Gentamycin.

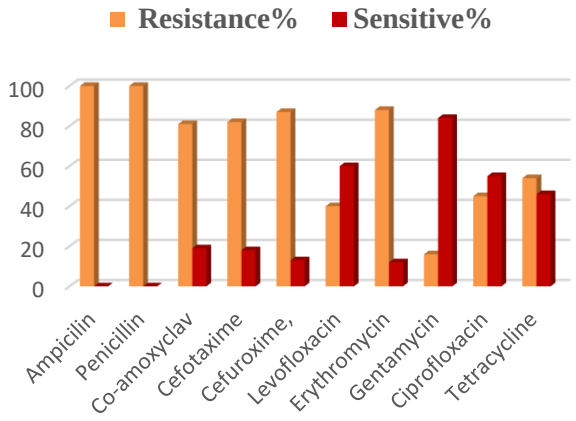

Figure: 5 Antibiotics sensitivity pattern of *S. pyogenes*

Antibiotics sensitivity pattern *of M. catarhalis:*M. catarhalis exhibited 100% resistance and 0% sensitivity against Ampicillin followed by 98% resistance and 2% sensitivity against co. amoxiclave, Cefuroxime 89% resistance and 11% sensitive, Cefotixime 86% resistance, 85% resistance and 15 % susceptible to tetracycline and 16% sensitive, 60% resistance and 40% sensitive to Gentamycin.

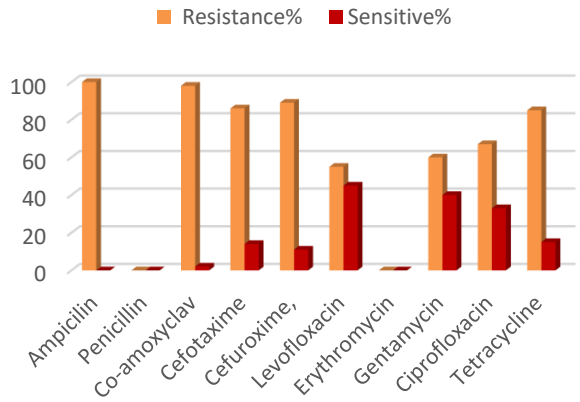

Figure: 6 Antibiotics sensitivity pattern *of M. catarhalis*

Antibiotics sensitivity pattern of H. *influenza*: *H. influenza* was observed 100 % resistance and 0% sensitive to Ampicillin followed by Co. amoxiclave 98% resistance and 2% sensitive, Cefuroxime 90% resistant and 10% sensitive, 87% resistant and 13% sensitive to Tetracycline. While against Cefotixime 80% resistance and 20 % sensitive. The bacteria *H. influenza* susceptible to Levofloxacin 85% and 15% resistance followed by Gentamycin 20% resistance.

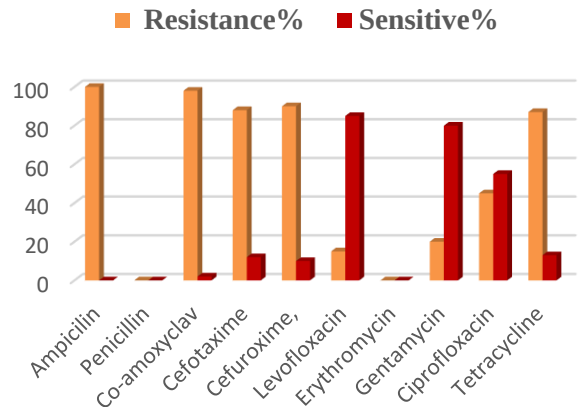

Figure: 7 Antibiotics sensitivity pattern of *H. influenza*

Antibiotics sensitivity pattern of *Enterococcus Species*: Ampicillin was noted99% resistance and 1% sensitive to *Enterococcus Species* followed by Penicillin against 97% resistance and 3% sensitive, 86 resistance and 16% sensitive to Cefuroxime, 79% resistant and 19% sensitive to Cefotixime, 78% resistant and 22% susceptible C0-amoxiclave, 54% resistant and 46% sensitive to Tetracycline, 56% resistant and 44% sensitive to Tetracycline, 20% resistant and 80%% sensitive to Ciprofloxacin, 20% resistant and 80% sensitive to Gentamycin, 16% resistance and 84% sensitive to Levofloxacin.

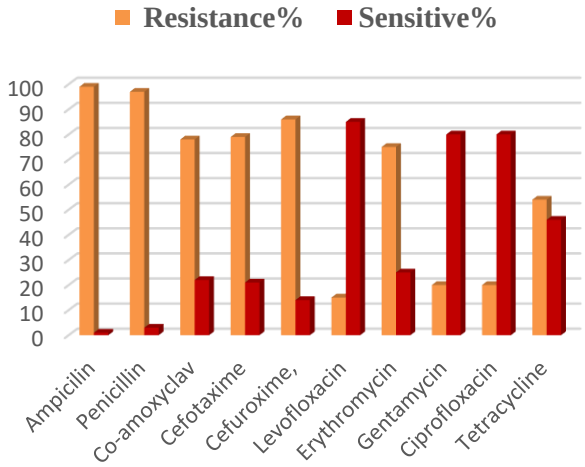

Figure: 8 Antibiotics sensitivity pattern of *Enterococcus Species*

Antibiotics sensitivity pattern of *S. aureus*: Ampicillin was observed 100% resistance and 0% sensitive to *S. aureus* followed by Penicillin against 98% resistance and 2% sensitive, 80 resistance and 20% sensitive to Cefuroxime, 78% resistant and 22% sensitive to Co-amoxiclave, 76% resistant and 24% sensitive to Cefotixime, 69% resistant and 31% sensitive to Erythromycin, 56% resistant and 44% sensitive to Tetracycline, 18% resistant and 82%%

sensitive to Ciprofloxacin, 16% resistant and 84% sensitive to Levofloxacin and 16% resistance and 84% sensitive to Gentamycin.

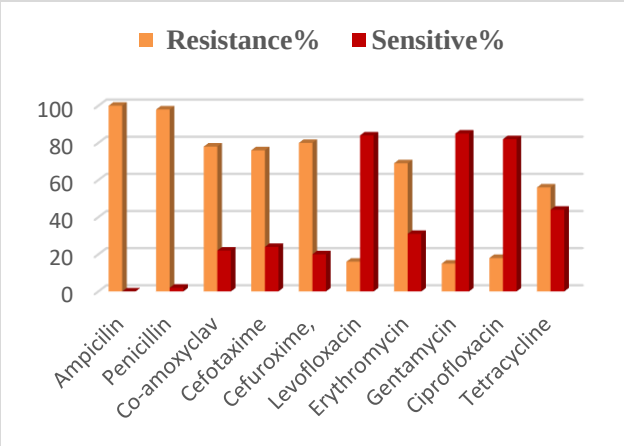

Figure: 9 Antibiotics sensitivity pattern of *S. aureus*

The average antibiotics resistance were: ampicillin was 99.7% resistance to all antibiotics, followed by penicillin 98.2%, Co-amoxiclave 88%, Cefuroxime 86%, Cefotixime 82%, Tetracycline 67.7%, Ciprofloxacin 50.4%, Levofloxacin 46.2%, Erythromycin 43% and Gentamycin 37%.

Discussion

In the current study, different bacterial species were investigated in respiratory tract infection followed by their identification and checked the antibiotic susceptibility profile of isolated bacteria. Nowadays the resistance produced by microbes (especially bacteria) is one of the foremost health issues in the developing world. An alternate therapy must be cost effective to stop this drug resistance over and above (Keith et al., 2010; Shrestha et al., 2013). Our study comprises 130 sputum and 70 throat swabs investigated for screening pathogenic bacteria. The bacterial isolates from the swabs of the upper respiratory tract were S. aureus (42.5%) followed by *S. pyogenes (*20.3%), *S. pneumoniae* (16.5%), *K. pneumoniae*(11.5%), *M. catarhhalis* (8.7%). While bacteria in the sputum of the lower respiratory tract were *coliform*s (40.4*%), P. aeruginosa* (29.4%) *S. pneumoniae* (11.5%), *H. Influenza (6.5%), M. catarhalis*(4.6%). Our result is in comparison with the Atia *et al.* (2018), who also investigated the pathogenic bacteria of URTIs. Of 530 processed samples, 80.5% showed positivity, wherein pathogenic bacteria were identified in 83.7% of sputum samples and 56.5% of throat swabs. Most frequent bacterial specie was *S. pneumoniae* (48%), followed by *P. aeruginosa* (23%), *S. aureus* (13%), *Enterobacter* (8%), *C. freundil* (5%), and latest with *Klebsiella* (3%).

In our study 130 sputum from LRTIs were analyzed and noted bacterial species in sputum were *coliform*s (40.4*%), P. aeruginosa* (29.4%) *S. pneumoniae* (11.5%), *H. Influenza (6.5%)M. catarhalis*(4.6%). In contrast to the result described by Amarasinghe et al. (2018) who presented similar results showing 29.4% positive results for pathogenic bacterial organisms. Coliforms, 43.6%, and *P. aeruginosa* 29% were the most commonly isolated bacteria, followed by *Moraxella* 11.6%, *H. influenzae* (*n* = 23, 5.7%), and *S. pneumoniae*, 4.4% (Agmy et al., 2013). Among the pathogenic bacteria colonized in the respiratory tract, MDR is a severe problem, particularly those causing hospital-acquired infections. When different antibiotics were tested against isolated bacterial pathogens it was found that *S. pneumonia* resistance to Ampicillin, Penicillin, Co-amoxyclav, Cefotixime, Cefuroxime, 99%, 98%, 85%, 78%, 82% respectively, while low resistivity were determined against Ciprofloxacin 30%, Levofloxacin 25% and Gentamycin 18%. A similar study was conducted by Dutta and his colleagues, who reported that the isolates of *S. pneumonia* also exhibited higher susceptibility to cefuroxime (93.7%) (Dutta et al., 2017; Uzoamaka et al., 2017). The representation of the susceptibility of Penicillin-G, Ciprofloxacin, Tetracycline, and Erythromycin amounted 14.17%, 14.66%, 13.92%, and 8.16% respectively (Karaiskos and Giamarellou, 2014; Karcic et al., 2015).

In our study *K. pneumoniae* was observed 100 resistance to Ampicillin and penicillin followed by Co- Amoxiclave 98%, Cefotixime 85%, Cefuroxime 90%, Ciprofloxacin and Gentamycin 78% and 75% respectively. The current findings were compared with the work done by Malik *et al.*, (2019), in which *S. pneumoniae* was 66.7 % resistant to penicillin and 100% sensitive to Cefuroxime. Agmy and his colleagues explained that antimicrobial resistance among *S. pneumonia* has risen dramatically over the past years. By the early 1990s, penicillin-resistant clones of *S. pneumoniae* spread rapidly across the world (Agmy et al., 2013). In our investigation *S. pyogenes* were noted in higher resistance 100% against Ampicillin and Penicillin followed by Cefuroxime, Cefotixime, and Co-amoxiclave 87%, 82% and 81% respectively. Results were compared with the study of Dutta *et al*., (2017) investigated the higher sensitivity of *S. pyogenes* against cefuroxime (90%), levofloxacin (90%), gentamycin (80%) followed by amoxyclav (75%) and penicillin (70%). *H. influenza* was noted in maximum resistance to levofloxacine15% followed by Gentamycin 20% and Ciprofloxacin 45% While higher resistance to Ampicillin and penicillin. Wang and his colleagues gave a similar result that resistance rates of the HI isolates to cefuroxime, Cefotixime were 31.2%, 5.9%, respectively and suggested that the main mechanism used by *H. Influenza* to resist ampicillin is the production of β-lactamase to break antibiotics (Wang et al., 2019).

Conclusion

In the study, 130 sputum and 70 throat swabs were tested for respiratory bacterial pathogens. The samples were positive for different bacterial pathogens. Positive respiratory samples, *enterococcus*

showed higher frequency (29.3%*)* followed by *S. aureus* (27.4%), *S. pneumoniae* (14.0%), and M. *catarhalis (8.4%). K. pneumoniae* (7.3%), *S. pyogenes* (7.9%), *H. Influenza* (6.1%) showed lowest frequency. Antibiotics susceptibility profile was also done to determine resistance levels in isolated species against current antibiotics. Antibiotics susceptibility profile was done to determine resistance level in isolated species against current antibiotics. Few antibiotics were effective, while others were ineffective, and multi-drug resistance bacteria were detected. Further molecular research is needed to identify resistance genes among these species.

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Declaration

Data Availability statement

All data generated or analyzed during the study have been included in the manuscript.

Consent for publication

Not applicable

Funding

There were no sources providing support, for this research.

Conflict of interest

The authors assure that there were no financial relationships involved that could be perceived as a conflict of interest.

Acknowledgment

The authors are very thankful to the Abasyn University Peshawar, Khyber Pakhtunkhwa.

Author, Disclosure Statement

Ethics approval and consent to participate

The study permitted by the Ethical Committee of Abasyn University Peshawar and written informed consent was obtained from all the patients participated in the study.

Author Contributions

Abdullah did the experiments and wrote the manuscript. FUA, NH and BK conceived the study and design, AAK, BH and IM, SK, KN, ZU reviewed the manuscript. All the authors participated in the experimentation & optimizations.