Bacteriological Profile And Antibiogram Pattern In Patients With Respiratory Tract Infection

Bhandari R[1,2], Rana B[2], Chaudhary A[2], Pandey M[2], Shrestha G[2], Regmi R[1], Khadka R B[2], Kari K D[2], Neupane B[2]
1. Department of Microbiology, Devdaha Medical College & Research Institute, Devdaha, Nepal
2. Department of Microbiology, Crimson College of Technology, Butwal, Nepal

ABSTRACT

Introduction: Globally, respiratory tract infections are serious infectious disease affecting the organ of respiratory tract. A distinct group of microbes including bacteria, virus, fungus and parasite are associated with respiratory tract infection and is the leading cause of high morbidity and mortality. This study was conducted to determine the prevalence of bacteria associated with respiratory with antimicrobial susceptibility testing.

Methods: This is a hospital-based cross-sectional study conducted between March, 2022 and June, 2023 among patients who visited Crimson Hospital of Butwal with respiratory tract infection. Bargery's standard procedure has been followed for the isolation and identification of bacteria. Antimicrobial susceptibility testing was performed based on the Clinical & Laboratory Standard Institute (CLSI) guidelines.

Results: Out of 358 only 186 (52.05%) respiratory samples showed bacterial growth. A total of 332 bacterial isolates were identified, comprising 203 (61.1%) Gram-positive and 129 (38.85%) Gram-negative bacteria. Among gram-positive isolates *Staphylococcus aureus* (30.7%, n=102) and *Coagulase-Negative Staphylococcus* (25.6%, n=85) were the most predominant. In contrast, *Escherichia coli* 13.2% (44) and *Klebsiella pneumoniae* 9.6% (32) were the predominant gram-negative bacteria isolates. Gram-positive bacterial isolates exhibited complete sensitivity to Vancomycin and Linezolid (100%), while, gram-negative bacterial isolates showed considerable sensitivity to Tetracycline (82.9%) and Cefuroxime (75%)

Conclusion: A high prevalence of respiratory tract infection caused by a distinct group of bacterial pathogens has been reported. High number of Methicillin resistant *Staphylococcus aureus* (MRSA), Extended Spectrum Beta-lactamase (ESBL), and Multi-drugs Resistant (MDR) producing bacteria were detected.

Keywords: Antibiogram; Bacterial pathogen; Pneumonia; Respiratory tract infection

INTRODUCTION

Respiratory tract infections (RTI) are the most prevalent microbial infection in humans across the world. The incidence of RTI is increasing, particularly in developing nations like Nepal.[1-3] In accordance with the 2016, Nepal Demographic and Health Survey (NDHS), 1,750,688 cases of acute respiratory tract infection (ARTI) have been reported in Nepal. Both upper-respiratory tract infection (URTI) and Lower-respiratory tract infection (LRTI) are prevalent; however, URTI is is the more prevalent; but in terms of severity of disease, LRTI is more severe. In 2019, the global incidence rate of URTI was 17.2 billion, of which 42.8% died.[4] The global incidence of LRTIs among patients worldwide is 48.9 million, of which 1.5 million died.[1,5] A high incidence of URTI and LRTI has been reported among elderly patients above 70 years and children less than 5 years but is least prevalent among the young and adult population.[6,7] Distinct group of microbial pathogens are associated with RTI. About 70% of URTIs are caused by viruses and about 60-70% of cases of LRTIs are caused by bacteria.[8] A distinct group of bacteria, including *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Staphylococcus aureus, Klebsiella spp., and Pseudomonas spp.,* are frequently associated with RTI.[9,10] At present,

Corresponding author:
Dr. Ramesh Bhandari (Ph.D)
Department of Microbiology
Devdaha Medical College & Research Institute
Email: rameshbhandari2017@yahoo.com
Phone no: +9779849827997

irrational use of antimicrobial agents and inappropriate antibiotic therapy cause the emergence of multidrug-resistant respiratory pathogens, which increases hospital stay, mortality rate and healthcare-related expenditure for patients.[11,12] The present study aimed to determine the prevalence and antimicrobial susceptibility pattern of respiratory bacterial pathogens in Crimson Hospital, Nepal.

METHODS

This is a hospital-based cross-sectional study conducted at the Department of Microbiology of Crimson Hospital, Butwal, Nepal, from June 2022 to May 2023. Ethical clearance was obtained from the Ethical Review Committee of Pokhara University, Kaski, Nepal, with a reference number RF.No.206/2080/81. All the patients attending at Crimson Hospital with complaints of sore throat, pharyngitis, laryngitis, tonsillitis, bronchitis, bronchiolitis, and pneumonia were enrolled. The patients suspected with RTI with asthma, and pre-existing chronic illness likes lungs disease, dysphagia and patients on or had corticosteroid were excluded because their symptoms could mimic respiratory tract infection.

A convenient sampling method was used. A total of 358 patients were enrolled, and the size of participants was determined using the following formula.

$n = Z^2 p (1-p)/d^2$, where

Z is confidence interval CI (Z=1.96 for 95% CI)

P = prevalence which we have considered 37% from the previous study of Bhurtel. R et al[13]

d: The margin of error was considered 5% where d = 0.05, the formula used to calculate the sample size (n) was as follows.

$n = (1.96)^2 \times 0.37 (1-0.37)/0.0025 = 358$

Bacterial isolation and identification:

Respiratory samples such as throat swabs and sputum were collected. The throat swab was obtained by passing sterile cotton swab over the surface of the tonsillar fossa and posterior pharynx. Additionally, deep expectorated cough (sputum) was collected by patients themselves directly coughing in sterile container. Respiratory samples were directly inoculated onto 5% sheep blood agar (HiMedia, USA), MacConkey agar (HiMedia, USA), and Chocolate agar (HiMedia, USA) incubated overnight at 37°C in a 5 – 10% CO_2 incubator. Pure bacterial isolates in distinct culture media were identified on the basis of gram-staining, colony characteristics and distinct biochemical test including Catalase, Coagulase, Oxidase, Indole, Methyl Red, Voges-Proskauer (VP), Urease, Citrate utilization, Triple sugar iron agar (TSI), Sulphide-Indole Motility test (SIM) test as mentioned in Bergeys Manual of Systematic Bacteria and Clinical and Laboratory Standards Institute (CLSI, PA, USA) guidelines 2017.[14,15]

Antimicrobial Susceptibility Test (AST):

A modified Kirby-Bauer disc diffusion method was used to perform the antimicrobial susceptibility test on Mueller-Hinton Agar (Hi-Media, USA) as per CLSI guidelines.[15] A homogenous bacterial cell suspension was prepared by mixing 3-5 identical colonies from a pure culture plate into sterile nutrient broth (Himedia, USA) and adjusting the turbidity to 0.5 McFarland. A sterile cotton swab soaked in bacterial cell suspension was used to seed bacteria over the surface of Muller-Hinton agar (MHA). A specific antimicrobial disc was placed using sterile forceps and incubated overnight at 37°C. ZOI was measured and interpreted for sensitive, resistance and intermediate as per CLSI guidelines. Following antibiotics discs, Amikacin (AK, 30 µg), Ampicillin (AMP, 10 µg), Azithromycin (AZM, 15 µg), Cefoxitin (CXT, 30µg), Trimethoprim/sulphmethoxozole (SXT, 25 µg), Ciprofloxacin (CIP, 5µg), Clindamycin (CD, 2 µg), Cefixime (CFM, 5 µg), Cefuroxime (CXM, 30 µg), Gentamicin (GEN, 30 µg), Levofloxacin (LE, 5 µg), Linezolid (LZ, 30 µg), Oxacilin (OX, 1 µg), Penicillin G (P, 10 units), Tetracycline (TE, 30 µg), Vancomycin (VA, 30 µg), Ceftazidime (CAZ, 30 µg), Cefotaxime (CTX, 30 µg), Imipenem (IMP, 10 µg), Meropenem (MRP, 10 µg), and Ceftriaxone (CTR, 30 µg) of antibiotics of Himedia was used as per the CLSI guidelines and their availability and frequent prescription by physicians for treatment of the RTI.

Screening and confirmation of MRSA producers:

MRSA screening was done using oxacillin (1µg). Any *Staph. aureus* isolates producing a Zone of Inhibition (ZOI) <18 mm around the oxacillin disc were suspected MRSA producers. Phenotypic confirmation of MRSA was done using a cefoxitin

disk (30 µg). Any suspected MRSA producers producing the ZOI <17 mm around the Cefoxitin disk (30µg) were confirmed as *Methicillin Resistant Staphylococcus aureus (MRSA)*.

Screening and confirmation of Extended-Spectrum Beta-lactamase (ESBL) producers:
Preliminary screening of ESBL production was done using one of these 3rd-generation cephalosporin antibiotics. Any GNB isolates resistant to one of the 3rd generation cephalosporin antibiotics i.e., CTX 30µg, CTR 30µg, and CAZ 30µg, were considered as suspected ESBL producers. ESBL-producing isolates were phenotypically confirmed using the combined disc method. The ZOI were assessed individually for the CAZ 30 µg and CTX 30 µg discs, as well as in combination with clavulanic acid (10 µg), with the discs positioned 25 mm apart (center to center). Isolates showing a zone size increase of 5 mm or more around one or both of the clavulanate combined discs compared to the antibiotic alone were confirmed as ESBL producers.

Screening of MDR:
Microorganisms demonstrating resistance to three or more classes of antibiotics were considered as multidrug resistance.[16] In this study, five class of antibiotics were chosen.

Quality Controls:
Microbiology standards operating procedure was strictly followed throughout the process. For quality assurance, ATCC strains of *Streptococcus pneumoniae ATCC® (700603), E. coli ATCC® 25922,* and *Pseudomonas aeruginosa strains* (ATCC 27853) were used.

Statistical analysis:
Data obtained from the study was entered in Microsoft Office Excel 2016 and then exported to Graph Pad Prism Version 8.4.2 (789) Boston, MA 02110, USA for statistical analysis. Descriptive qualitative analysis findings were demonstrated in frequencies, percentages, cross-tabulation, bar charts, and pie chart.

RESULTS

Study Participants:
A total of 358 were enrolled in this study, with a 52% (n = 186) female and 48% (n = 172) male. Participants in this study varied in age from 0 to 80 above, with a mean age group of 45 ± 0.6 years and a median age of 43 ± 0.6 years. The majority of participants actively participated in this study were aged 21 to 40 years (n = 130, 36.43%).

Table 1: Demographic and clinical characteristics of participants with respiratory tract infection

Participants Frequency (N=358)	
Sex	n (%)
Female	186 (52.05%)
Male	172 (48%)
Toral	358
Age wise Distribution of patient in Year	
0-20	28 (7.82%)
21-40	130 (36.43%)
41-60	111 (30.65%)
61-80	73 (20.5%)
81 above	16 (4.38%)
Age (mean±SD), Years	45.6 ±52.84
Age (median)±, Years	43 ± 0.6

Bacteria Isolation and Their Prevalence:
Of the 358 clinical samples, 186 (52%) showed positive growth, 22% (n = 41) samples had monomicrobial growth and 78% (n = 145) had polymicrobial bacterial growths (Figure 1A). A total of 332 distinct bacteria have been isolated from 186 positive bacterial growth (Figure 1B). Female respiratory samples yielded the most bacteria i.e., 186 (52%), followed by males 172 (48%) respectively (Table 1). Among the respiratory samples, a significant number of bacteria have been recovered from sputum. Although both gram-positive and gram-negative bacteria have been recovered, gram-positive cocci were the most prevalent bacterial isolate, accounting for 61.1% (n = 203), followed by gram-negative bacilli 38.8% (n = 129) (Table 2). *Staphylococcus aureus* was the most prevalent gram-positive cocci (GPC) isolate, accounting for 30.7%, followed by *Coagulase-negative Staphylococcus* (CoNS) (25.6%), *Streptococcus pyogenes* (3.91%), and *Streptococcus pneumoniae* (0.9%) (Table 2 & Figure 2). Similarly, among the gram-negative bacterial isolates, *Escherichia coli* was the predominant isolates, accounting for 13.2% (n=44) followed by Klebsiella pneumoniae 9.6% (n = 32), Acinetobacter spp.,6.2% (n = 20), Pseudomonas spp., 5.12% (n = 20), Citrobacter spp.,3.01% (n = 10), and Morganella spp.,1.8% (n = 6) (Table 2 & Figure 2).

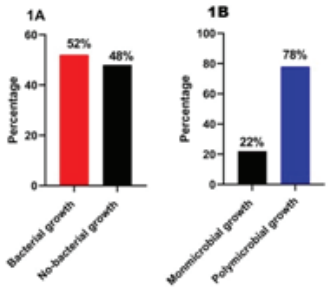

Figure 1. **1A** shows the bacterial growth rates and **1B** shows bacterial growth types from respiratory samples

Table 2: Isolation of bacteria from respiratory samples (Throat swab & Sputum)

Pathogen type (N/%)	**Microorganism**	**Bacteria Isolation From**		**Total Isolates**
		Throat swab n=18	**Sputum n=168**	
Gram-negative bacilli (N/%) (129/38.85%)	Escherichia coli	0	44	44 (13.2%
	Klebsiella pneumoniae	3	29	32 (9.6%)
	Acinetobacter species	0	20	20 (6.2)
	Morganella species	0	6	6 (1.8)
	Citrobacter species	0	10	10 (3.01%)
	Pseudomonas species	2	15	17 (5.12%)
Gram-positive cocci (N/%) (203/ 61.11%)	Staphylococcus aureus	4	98	102 (30.7%
	CONS	7	78	85 (25.6%)
	Streptococcus pyogenes	2	11	13 (3.91%)
	Streptococcus pneumoniae	0	3	3 (0.9%)
	Total	**18 (9.6%)**	**168 (90.32%)**	**332 (100%)**

Abbreviations: CoNS: (Coagulase Negative Staphylococcus)

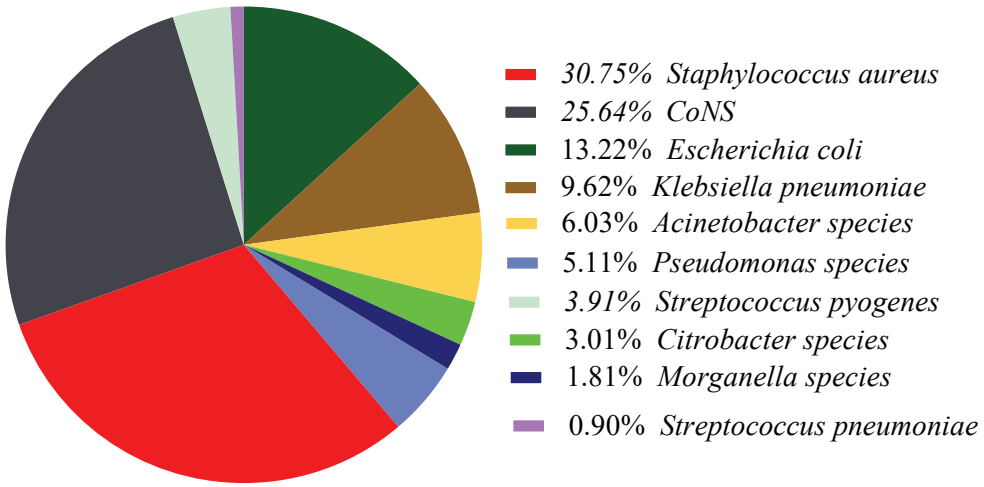

Figure 2. Percentile distribution of bacteria in positive respiratory samples from the patients with RTI

Antibiogram profile of gram-positive bacterial isolates:

Gram-positive bacterial isolates showed 100% sensitive to vancomycin and linezolid, but high degree of resistant to penicillin G (87.2%), ampicillin (72.9%), and amikacin (70.45%), While considering an individual gram-positive bacteria *S. aureus* exhibits 100% of sensitivity against vancomycin & linezolid and a higher degree of resistance against penicillin G (92.15%), ampicillin (88.34%), and amikacin (83.33%).

Similarly, CONS also exhibited 100% sensitivity against vancomycin and linezolid and a higher degree

of resistance against penicillin-G (75.29%), followed by ampicillin (62.35%) and amikacin (62.35%). *Streptococcus species* showed a higher level of sensitivity to vancomycin and linezolid; in contrast, it showed a higher resistance against clindamycin and azithromycin (Table 3).

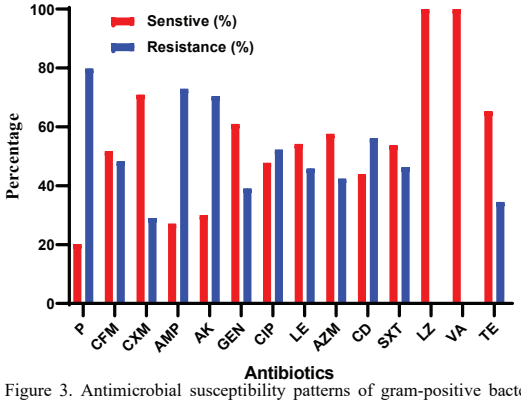

Figure 3. Antimicrobial susceptibility patterns of gram-positive bacterial isolates.
Abbreviations: P: Penicillin G, **CFM:** Cefixime, **CXM:** Cefuroxime, **AMP:** Ampicillin, **Ak:** Amikacin, **GEN:** Gentamicin, **CIP:** Ciprofloxacin, **LE:** Levofloxacin, **AZM:** Azithromycin, **CD:** Clindamycin, **SXT:** Trimethoprim-sulfamethoxazole, **LZ:** Linezolid, **VA:** Vancomycin, **TE:** Tetracycline.

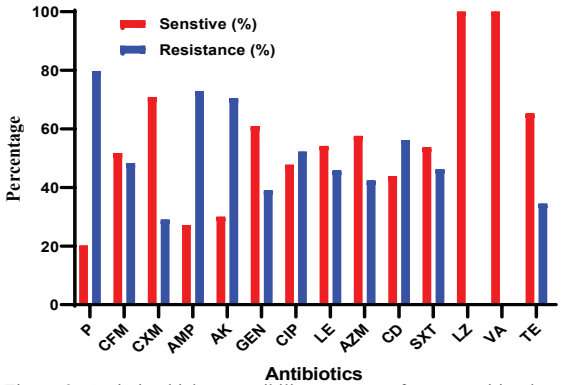

Figure 3. Antimicrobial susceptibility patterns of gram-positive bacterial isolates.
Abbreviations: P: Penicillin G, **CFM:** Cefixime, **CXM:** Cefuroxime, **AMP:** Ampicillin, **Ak:** Amikacin, **GEN:** Gentamicin, **CIP:** Ciprofloxacin, **LE:** Levofloxacin, **AZM:** Azithromycin, **CD:** Clindamycin, **SXT:** Trimethoprim-sulfamethoxazole, **LZ:** Linezolid, **VA:** Vancomycin, **TE:** Tetracycline.

Table 3. Antimicrobial susceptibility pattern of gram-positive cocci isolates

Antibiotics	Pathogens												Total			
	Staphylococcus aureus				CONS				Streptococcus spp.,							
	S		R		S		R		S		R		S		R	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
P	8	7.84	94	92.15	21	24.70	64	75.29	12	75.0	4	25.0	41	20.19	162	79.80
CFM	47	46.07	55	53.92	49	57.64	36	42.35	9	56.25	7	43.75	105	51.72	98	48.27
CXM	68	66.66	34	33.33	64	75.2	21	24.8	12	75.0	4	25.0	144	70.93	59	29.06
AMP	12	11.65	90	88.34	32	37.64	53	62.35	11	68.75	5	31.25	55	27.09	148	72.90
AK	17	16.6	85	83.33	32	37.64	53	62.35	12	75.0	4	25.0	61	30.0	143	70.45
GEN	62	60.78	40	39.21	52	61.17	33	38.82	NA	NA	NA	NA	114	60.96	73	39.03
CIP	28	27.45	74	72.54	56	65.88	29	34.1	13	81.25	3	18.75	97	47.78	106	52.22
LE	36	35.29	66	64.70	60	70.58	25	29.4	14	87.5	2	12.5	110	54.18	93	45.82
AZM	54	52.94	48	47.05	58	68.23	27	31.7	5	31.25	11	68.75	117	57.63	86	42.37
CD	31	30.39	71	69.60	52	61.17	33	38.8	6	37.5	10	62.5	89	43.84	114	56.16
SXT	56	54.90	46	45.09	46	54.11	39	45.88	7	43.75	9	56.25	109	53.69	94	46.30
LZ	102	100	-	-	85	100	-	-	14	100	-	-	203	100	-	-
VA	102	100	-	-	85	100	-	-	14	100	-	-	203	100	-	-
TE	54	52.90	48	47.05	68	80	17	20.0	NA	-	NA	-	122	65.24	65	34.47

Abbreviations: P (Penicillin G), **CFM** (Cefixime), **CXM** (Cefuroxime), **AMP** (Ampicillin), **AK** (Amikacin), **GEN** (Gentamycin), **CIP** (Ciprofloxacin), **LE** (Levofloxacin), **AZM** (Azithromycin), **CD** (Clindamycin), **SXT:** Trimethoprim sulfamethoxazole), **LZ** (Linezolid), **VA** (Vancomycin), **TE** (Tet- racycline)

Antibiogram of Gram-negative bacterial isolates:

In this study, gram-negative bacteria are highly sensitive to both tetracycline (82.94%) and cefuroxime (75.96%). Conversely, GNB isolates exhibit substantial level of resistance to ciprofloxacin (51.94%) and amikacin (60%) (Figure 4 & Table 4). Antibiogram of individual gram-negative bacilli showed, E. coli and Klebsiella pneumoniae were highly sensitive to tetracycline and highly resistant to amikacin. Likewise, Acinetobacter species, exhibited significant resistant to amikacin (70%) and high susceptibility to ceftazidime (80 %) and cefuroxime (80%). Morganella species were 100% sensitive to cefotaxime, ceftazidime, and cefuroxime and, 70% resistance to

Table 4. Antimicrobial susceptibility pattern of Gram-Negative Bacterial Isolate

	Pathogens													
	E. coli		Klebsiella pneumoniae		Acinetobacter species		Morganella species		Citrobacter species		Pseudomonas species		Total	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
AK	18 (40.9)	26 (59.1)	13 (40.6)	19 (59.3)	6 (30.0)	14 (70.0)	3 (50)	3 (50)	4 (40)	6 (60)	7 (41.1)	10 (59.9)	51 (39.5)	78 (60.4)
GEN	25 (56.8)	19 (43.1)	27 (84.3)	5 (15.6)	14 (60.0)	6 (40.0)	4 (66.6)	2 (43.4)	5 (50)	5 (50)	12 (70.5)	5 (29.5)	87 (67.4)	42 (32.5)
IMP	28 (63.6)	16 (36.3)	18 (56.2)	14 (43.7)	9 (45.0)	11 (65.0)	5 (83.3)	1 (16.7)	6 (60)	4 (40)	9 (52.9)	8 (47.1)	75 (58.1)	54 (41.8)
MRP	36 (81.1)	8 (18.8)	24 (75.0)	12 (25.0)	12 (60.0)	8 (40.0)	5 (83.3)	1 (16.7)	7 (70)	3 (30)	11 (64.7)	6 (35.3)	95 (73.6)	34 (26.3)
CIP	21 (47.7)	23 (52.2)	15 (46.8)	17 (53.1)	11 (55.0)	9 (45.0)	4 (66.6)	2 (43.3)	4 (40)	6 (60)	7 (41.1)	10 (59.9)	62 (48.0)	67 (51.9)
LE	22 (50.0)	22 (50.0)	18 (56.2)	14 (43.7)	12 (60.0)	8 (40.0)	3 (50.0)	3 (50.0)	4 (40)	6 (60)	9 (52.9)	8 (47.1)	68 (52.7)	61 (47.2)
CAZ	33 (75.0)	14 (25.0)	22 (68.7)	10 (31.2)	16 (80.0)	4 (20.0)	6 (100)	0 (0)	8 (80)	2 (20)	12 (70.5)	5 (29.5)	97 (75.2)	32 (24.8)
CTX	31 (70.4)	13 (29.5)	20 (62.5)	12 (37.5)	15 (75.0)	5 (25.0)	6 (100)	0 (0)	10 (100)	0 (0)	12 (70.5)	5 (29.5)	94 (72.8)	35 (27.1)
CTR	31 (70.4)	13 (29.5)	20 (62.5)	12 (37.5)	15 (75.0)	5 (25.0)	6 (100)	0 (0)	10 (100)	0 (0)	12 (70.5)	5 (29.5)	94 (72.8)	35 (27.1)
CXM	33 (75.0)	14 (25.0)	21 (65.6)	11 (37.3)	16 (80.0)	4 (20.0)	6 (100)	(0)	9 (90)	1 (10)	13 (76.4)	4 (23.6)	98 (75.9)	31 (24.0)
SXT	19 (43.1)	25 (51.8)	14 (43.7)	18 (56.2)	8 (40.0)	11 (60.0)	4 (66.6)	2 (43.4)	4 (40)	6 (60)	7 (41.1)	10 (59.9)	52 (40.3)	77 (59.60
TE	38 (86.3)	6 (13.6)	28 (87.5)	4 (12.5)	15 (75.0)	5 (25.0)	5 (83.3)	1 (16.7)	7 (70)	3 (30)	14 (82.3)	3 (17.2)	107 (82.9)	22 (17.0)

Abbreviations: AK: Amikacin, **GEN:** Gentamycin, **IMP:** Imipenem, **MRP:** Meropenem, **CIP:** Ciprofloxacin, **LE:** Levofloxacin, **CAZ:** Ceftazidime, **CTX:** Cefotaxime, **CTR:** Ceftriaxone, **CXM:** Cefuroxime, **SXT:** Trimethoprim-sulfamethoxazole, **TE:** Tetracycline

amikacin. Citrobacter species exhibit 100% sensitivity to cefotaxime and ceftriaxone and 60% resistance to amikacin, ciprofloxacin, and levofloxacin. Pseudomonas species were extremely sensitive to tetracycline (82.3%) and exceptionally resistant to amikacin, ciprofloxacin and trimethoprim sulfamethoxazole (59.9%) (Table 4).

Prevalence of MRSA & ESBL Producers

Of the 102 S. aureus, 68.62% (70) were suspected of MRSA production in the preliminary screening test of MRSA. However, only 49.01% (50) confirmed as MRSA producers by the phenotypic disc diffusion method (Figure 5A). Similarly, 27.13% (35) of the total 129 GNB isolates were suspected of ESBL production. Meanwhile, only 13.95% (18) confirmed as ESBL producers by the phenotypic double disc diffusion method (Figure 5B).

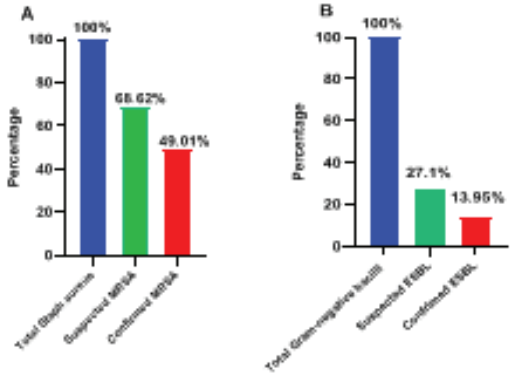

Figure 5. A and B showed the distribution of MRSA and ESBL producers respectively

Prevalence of Multidrug Resistance Bacterial Respiratory Pathogens:

67.46% (n=224) of the 332 bacterial isolates were

MDR. 59.6% (121) of the 203 gram-positive cocci isolates were multidrug resistant, meanwhile 85.5% (103) of the 121 gram-negative bacilli isolates, were multidrug resistant (MDR) (Table 5 & 6). A high proportion of Gram-negative bacterial isolates were found to be multidrug-resistant (MDR), including 90.6% (n = 29) of Klebsiella pneumoniae, 86.3% (n = 38) of Escherichia coli, 85% (n = 17) of Acinetobacter spp., and 82.35% (n = 14) of Pseudomonas spp Similarly, among the gram- positive bacterial isolates 68.63% (n=70) were Staph. aureus, 56.46% (48) were CoNS and 3.12% (5) were Streptococcus species (Table 5).

Table 5. Multidrug resistance pattern of gram-positive cocci

Resistance Patterns	**No of Antibiotics (Classes)**	**S. aureus (N=102)**	**CONS (N=85)**	**Streptococcus species (N=16)**	**Total (N=203)**
P, AMP, AK, CIP	3	4	6	2	12
P, AMP, AK, LE	3	5	3	1	9
P, AMP, AK, CIP	3	3	2	1	6
P, AMP, AK, LE	3	3	2	-	5
P, AMP, GEN, CIP	3	4	5	1	10
P, AMP, GEN, LE	3	6	3	-	9
P, AMP, AK, GEN, CIP, LE	3	6	4	-	11
P, AMP, CFM, AK, GEN, CIP, LE	3	7	4	-	11
P, AMP, CFM, Ak, GEN, AZM	3	3	3	-	6
P, AMP, CFM, AK, GEN, AZM, CD	3	4	3	-	7
P, AMP, CXM, AK, AZM, SXT	3	5	4	-	9
P, AMP, CFM, CXM, AK, GEN, SXT	4	7	3	-	10
P, AMP, CXM, CFM, GEN, TE, SXT	4	6	3	-	11
P, AMP, CFM, CXM, GEN, SXT, TE, AZM	5	4	2	-	6
P, AMP, CXM, CFM, GEN, AK, CD, AZM, TE, SXT	5	3	1	-	4
Total MDR (n/%)		70 (68.63%)	48 (56.46%)	5 (31.25%)	121 (59.60%)

Table 6. Multidrug Resistant Patterns of Gram-negative bacilli isolates

Resistance Patterns	**No of Antibiotics (Classes)**	**E. coli N(44)**	**Klebsiella spp N(32)**	**Acinetobacter spp N(20)**	**Morganella spp N(6)**	**Citrobacter spp N(10)**	**Pseudomonas spp N(17)**	**Total (N=129)**
AK, CIP, SXT	3	5	6	3	1	1	2	18
AK, GEN, LE, SXT	3	5	3	2	1	1	1	12
AK, GEN, CIP, LE, SXT	3	2	1	1	-	-	1	6
GEN, IMP, CIP	3	3	2	1	-	-	2	8
GEN, CIP, TE	3	2	1	1	-	-	1	3
AK, GEN, IMP, CIP	3	2	1	1	-	-	1	5
AK, GEN, MRP CIP, LE	3	1	1	-	-	-	-	1
GEN, MRP, CIP	3	2	1	1	-	-	1	3
AK, MRP, LE, CIP, CAZ	4	3	2	1	-	-	1	8
AK, MRP, LE, CIP, CTX, CAZ	4	2	1	1	-	-	1	5
AK, GEN MRP, IMP, LE, CIP, CTX, CAZ	4	2	1	1	-	1	-	4
GEN, IMP, CIP, CTX, CAZ	4	2	1	1	-	-	-	3

GEN, IMP, CIP, LE, CAZ, CTR	4	2	2	1	-	-	1	3
AK, IMP, CIP, CTR, CAZ, CTX	4	1	2	1	-	-	1	3
AK, IMP, LE, CTR, CAZ, CTX	4	1	3	1	-	-	-	2
AK, GEN, IMP, LE, CAZ, CTX, SXT	5	1	1	-	-	-	-	2
GEN, LE, CIP, SXT, CTR, TE	5	1	-	-	-	-	1	1
AK, GEN, MRP, IMP, LE, CIP, CAZ, CTX, CTR SXT	5	1	-	-	-	-	-	1
AK, TE, CIP, IMP, SXT	5	1	-	-	-	-	-	1
Total MDR (n/%)		**38 (86.36%)**	**29 (90.62%)**	**17 (85%)**	**2 (33.3%)**	**3 (30%)**	**14 (82.35%)**	**N=103 (85.15%)**

DISCUSSION

Respiratory tract infections (RTIs) are amongst the most common and diverse groups of infections in humans worldwide, with a prevalence rate of 22% to 25%.[17] In this study, 52.08% of respiratory samples showed bacterial growth, 47.92% did not. It might be due to respiratory tract infection of viral or fungi origin, or, due to inappropriate methods of collection, transportation and processing of respiratory samples might have significantly interferes bacteria growth and reduced the bacterial viability. Our study findings was significantly higher compared to previous study conducted in Western Nepal (30.2%), Southern Ethiopia (33.5%), Jimma, Ethiopia (40.3%), and Egypt (45%).[18-21] However, a numerous study conducted in Kenya (95.4%), South India 55.3% and China (55.4%) showed significantly high bacterial growth as compared to our study findings. This discrepancy might be due to variation in the study period, geographical differences, and the socioeconomic status of the study population.[22-25] This study showed that 90.32% of bacteria were isolated from the sputum samples, which was significantly higher than findings of Shrestha et al., (31%), Raghubanshi, B. R et al., (29.31%), Subedi D et al., (29.13%) and Kaundinnyayana S et al., (9.87%) in Nepal.[26,27,18,28] This is more likely due to contamination of the sputum with upper respiratory tract secretions and the normal microbial flora in upper respiratory tract. Similarly, our study reported that RTI was more prevalent in males (48%) as compared to females (44.09%). A similar type of study was conducted by Saxena. S. et al. (68%), Panda. S et al., (63.85%), and Tasnia Ahmed et al. (55.91%) reported, RTI more frequent in males in comparison to females which is consistent with our study findings.[29,30] Higher incidence of RTI in males may be due to distinct associated risk factors, including smoking, alcohol consumption, working in the field, cooking in wood, hormonal differences and a weakened humoral and cell- mediated immune response.[31,32] RTI infection increases with the advancement of age.[33] Our study revealed a 36.43% RTI among patients between 20 & 40 years, which was different from the study finding of Singh S et al., who they reported lowest RTI rate of 17.3%. This difference might be due to higher proportion of participants between 20 – 40 years.[34] In this study both the gram-negative bacilli and gram-positive cocci were isolated; however, gram-positive cocci isolation was as significantly high i.e., (61.1%) compared to (38.89%) gram- negative bacilli. A similar type of study conducted by Usman A et al., Parsad A et al. (66.67%), Saxena. S et al., (65.95%) reported significantly high isolation of GNB compared to our study findings. In our study, Staphylococcus aureus was the predominant gram-positive cocci pathogen causing RTI. It might be due their prevalence as the normal flora in respiratory systems and their roles in causing distinct respiratory tract infections.[35,36] A study conducted in India by Kousalya K et al. also reported *S.aureus* (45.61%) as a predominant causing RTI.[37] However, in a similar type of study conducted by the Felege Hiwot(35.9%) Jimma 12.8% and Arba Minch 11.8% reported S. pneumoniae as the predominant GPB causing RTI. In this study, Escherichia coli was the most prevalent gram-negative bacilli causing RTI.[17] It is found as most commonly in hospital environment and able

to cause the opportunistic respiratory tract infections in both inpatients and out-patients. Higher isolation of Escherichia coli may be associated with a distinct factor hematogenous dissemination of Escherichia coli from gastrointestinal or urinary tract, presence of Escherichia coli in respiratory tract in hospitalized patients and aspiration of from pharynx.[38] However, a number of studies conducted by Sherchan JB et al (39.5%), and Usman A et al (66.5%) reported K. pneumonia as the predominate pathogen causing RTI in their study.[39,40] In the meantime, Martin-Loeches et al. (18.4%) and Miriti, D.M et al (51.2%)reported as Pseudomonas aeruginosa as the most prevalent pathogen causing RTI.[16,41] This variation in the prevalence of microorganisms causing respiratory tract infection might due to several factors including their distribution on respiratory tract as normal flora, seasonal variation affecting bacterial isolation from respiratory samples, stundy population, and the host`s immune status.[42,43] In this study we reported that gram-positive bacterial isolates were highly sensitive against vancomycin (100%), linezolid (100%) which is similar to findings of Ragubanshi B R et al, and Yang. Li et al.[27,44] Higher degree of sensitivity against vancomycin & linezolid may be due to its use as a last resort of drugs for treatment.[45] Similarly, our study reported a low sensitivity rate of gram- positive cocci against penicillin G (73.9%) and amikacin (70.45%). A similar type of study conducted by Kousalya K et al. also reported a high level of resistance to penicillin.[37] A study conducted by Muhammad Ali et al. showed S. aureus were 100% sensitivity to ciprofloxacin, Gentamicin which is most likely different from their study.[46] Similarly, our study findings showed Gram-negative bacilli were highly sensitive to tetracycline (82.9%) and cefuroxime (75.96%), however less sensitive against amikacin (60.26%) and trimethoprim-sulfamethoxazole (59.69%). A study conducted by Goel. N et al., reported respiratory pathogen are highly sensitive against Tetracycline and highly resistant against amikacin and gentamicin.[47] A study conducted by Regasa et al. and Tasnia Ahmed et al, reported Gram- negative bacilli isolated as highly resistant to tetracycline which is completely contrasting from our study findings. Our study reported 49% MRSA which might be due to overuse of broad-spectrum cephalosporins antibiotics, poor implementation of antibiotics stewardship programs in hospital and long-term hospital stay.[48] A similar type of study conducted by Kateete et al. reported 45% MRSA, which is low, meanwhile, in a study Yang L et al. reported 68.62%, which is significantly high as compared to our finding.[49] In the present study, the overall magnitude of multidrug resistance (MDR) in GNB and GPB was 85.15% and 59.6% respectively which is significantly high compared to study findings of Yimer O et al which reported 54.6% MDR gram-negative bacilli and 50% MDR gram-positive cocci isolates.[50] It might be due to irrational use of the antibiotics as empirical therapy for RTI around in hospital settings and also the mutation in genes of pathogenic microorganisms resulting in MDR.[51]

CONCLUSION

This study revealed gram-positive cocci and gram-negative bacilli were associated with the respiratory tract infection. Staphylococcus aureus and E. coli were the predominant pathogens causing RTI. High prevalence of multidrug resistance (MDR) especially ESBL and MRSA producing respiratory pathogens were observed. Our study findings add to the growing evidence of emerging antibiotic resistance within hospital setting highlight the need for increased support for periodic surveillance to antibiotics, strict implementation of antibiotic stewardship and education.

LIMITATION

This study has a number of limitations. Our study is cross-sectional study; thus, this study does not allow us to observe the prevalence of the distinct microbes in distinct interval of time and their susceptibility pattern over a period of time. Our study used the traditional methods for screening and identification of bacteria; however, molecular methods are widely accepted for identification, characterization, and confirmation of bacterial pathogens. Our study focused on the bacteria causing the RTI; thus, we do not perform tests for the identification of fungi, viruses, and parasites, which might represent a potential bias.

Acknowledgements:

We are grateful to patients for participating in this study. And we are also thankful to all the labo-

ratory and hospital staffs for their cooperation in obtaining necessary data and related information.

Contributions: RB: analyzed data, edited the manuscripts, design the study, data analysis, AC, BR, MP and GS per- formed the experiment and collect the data.

Funding: CCT has funded this study as a grant for dissertation of the student.

REFERENCES

1. Age–sex differences in the global burden of lower respiratory infections and risk factors, 1990–2019: results from the Global Burden of Disease Study 2019. The Lancet Infectious Diseases. 2022;22(11):1626-1647. doi:https://doi.org/10.1016/S1473-3099(22)00510-2)
2. El-Koofy, N.M., El-Shabrawi, M.H., Abd El-alim, B.A., Zein, M.M. & Badawi, N.E. Patterns of respiratory tract infections in children under 5 years of age in a low– middle-income country. Journal of the Egyptian Public Health Association. 2022 Nov 7;97(1):22. doi:10.1186/s42506- 022-00118-0
3. Peltola, V. & Ruuskanen, O. Respiratory Viral Infections in Developing Countries: Common, Severe, and Unrecognized. Clinical Infectious Diseases. 2008;46(1):58-60. doi:10.1086/524020.
4. Jin X, Ren J, Li R, Gao Y, Zhang H, et al. Global burden of upper respiratory infections in 204 countries and territories, from 1990 to 2019. EClinicalMedicine. 2021 Jul 14; 37:100986. doi:10.1016/j. Eclinm.2021.100986.
5. Ashrafi-Asgarabad, A., et al. The burden of lower respiratory infections and their underlying etiologies in the Middle East and North Africa region, 1990–2019: results from the Global Burden of Disease Study 2019. BMC Pulmonary Medicine. 2023 Jan 1;23(1):2. doi:10.1186/s12890-022-02301-7.
6. Forouzanfar MH, Alexander L, Anderson HR, Bachman VF, Biryukov S,, et al. Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet (London, England). 2015 Dec 5;386(10010):2287-323. eng. doi:10.1016/s0140- 6736(15)00128-2.
7. Kolditz, M. & Ewig, S. Community-Acquired Pneumonia in Adults. Deutsches Arzteblatt international. 2017 Dec 8;114(49):838- 848. eng. doi:10.3238/ arztebl.2017.0838.
8. Salazar, F., Bignell, E., Brown, G.D., Cook, P.C. & Warris, A. Pathogenesis of Respiratory Viral and Fungal Coinfections. Clinical microbiology reviews. 2022 Jan 19;35(1): e0009421.doi:10.1128/cmr.00094-21.
9. Zhang L, Xiao Y, Zhang G, Li H, Zhao J., et al. Identification of priority pathogens for aetiological diagnosis in adults with community-acquired pneumonia in China: a multicentre prospective study. BMC infectious diseases. 2023 Apr 14;23(1):231. doi:10.1186/s12879-023-08166-3.
10. McCullers, J.A. The co-pathogenesis of influenza viruses with bacteria in the lung. Nature reviews. Microbiology. 2014 Apr;12(4):252-62. doi:10.1038/nrmicro3231.
11. Mahendra M, Jayaraj BS, Lokesh KS, Chaya SK, Veerapaneni VV., et al. Antibiotic Prescription, Organisms and its Resistance Pattern in Patients Admitted to Respiratory ICU with Respiratory Infection in Mysuru. Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine. 2018 Apr 22(4):223-230. Doi: 10.4103/ijccm. IJCCM_409_17.
12. Llor, C. & Bjerrum, L. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. Therapeutic advances in drug safety. 2014 Dec;5(6):229-41. doi:10.1177/2042098614554919.
13. Bhurtel, R., Pokhrel, R.P. & Kalakheti, B. Acute Respiratory Infections among Under-five Children Admitted in a Tertiary Hospital of Nepal: A Descriptive Cross-sectional Study. JNMA; journal of the Nepal Medical Association. 2022 Jan 15;60(245):17-21. eng. Epub 2022/02/25. doi:10.31729/jnma.6889.
14. Bergey, D.H. Bergey's manual of determinative bacteriology : a key for the identification of organisms of the class schizomycetes, (Baltimore : The Williams & Wilkins Company, 1923.https://search.library.wisc.edu/ catalog/999609836402121
15. Lewis Ii, J.S., Clinical & Laboratory Standards,

I. Performance standards for antimicrobial susceptibility testing, (Clinical and Laboratory Standards Institute, 2023). https://cir.nii.ac.jp/crid/1130578271562670753

16. Magiorakos, A.P., et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases.2012 Mar;18(3):268-81. doi:10.1111/j.1469-0691.2011.03570.x.
17. Fleming, D.W., Cochi, S.L., Hightower, A.W. & Broome, C.V. Childhood upper respiratory tract infections: to what degree is incidence affected by day-care attendance? Pediatrics. Pediatrics. 1987 Jan;79(1):55-60.
18. Subedi, D., et al. Antibiotic Susceptibility Profile of respiratory pathogens obtained at tertiary care hospital from western Nepal. Nepal Journal of Biotechnology. 2019 Dec;29;7(1):1-7. doi:10.3126/njb.v7i1.26944.
19. Gebre, A.B., Begashaw, T.A. & Ormago, M.D. Bacterial profile and drug susceptibility among adult patients with community acquired lower respiratory tract infection at tertiary hospital, Southern Ethiopia. BMC infectious diseases. 2021 May 13;21(1):440. eng. doi:10.1186/s12879- 021-06151-2.
20. Regasa, B., Yilma, D., Sewunet, T. & Beyene, G. Antimicrobial susceptibility pattern of bacterial isolates from community-acquired pneumonia patients in Jimma University Specialized Hospital, Jimma, Ethiopia. Saudi Journal for Health Sciences. 2015;4(1):59-64. doi:10.4103/2278-0521.151411.
21. El-Sokkary RH, Ramadan RA, El-Shabrawy M, El-Korashi LA, Elhawary A., et al. Community acquired pneumonia among adult patients at an Egyptian university hospital: bacterial etiology, susceptibility profile and evaluation of the response to initial empiric antibiotic therapy. Infection and drug resistance. 2018 Nov 21:2141-2150. doi:10.2147/idr.S182777.
22. Josphat, M., John, M. & Anthony, K. Antimicrobial susceptibility patterns of bacteria associated with upper respiratory tract infections in Kitui, Kenya. Ethiop Med J. 2017;55(2):121-7.
23. Ramana, K.V., Kalaskar, A., Rao, M. & Rao, S.D. Aetiology and Antimicrobial Susceptibility Patterns of Lower Respiratory Tract Infections (LRTI¡¯s) in a Rural Tertiary Care Teaching Hospital at Karimnagar, South India. American Journal of Infectious Diseases and Microbiology. 2013;1(5):101-105.
24. Luan, Y., Sun, Y., Duan, S., Zhao, P. & Bao, Z. Pathogenic bacterial profile and drug resistance analysis of community-acquired pneumonia in older outpatients with fever. The Journal of international medical research. Epub 2018 July 22. doi:10.1177/0300060518786915.
25. Vanker A, Nduru PM, Barnett W, Dube FS, Sly PD., et al. Indoor air pollution and tobacco smoke exposure: impact on nasopharyngeal bacterial carriage in mothers and infants in an African birth cohort study. ERJ open research. 2019 Feb;5(1). doi:10.1183/23120541.00052-2018.
26. U. Shrestha, A. Singh, B. M. Pokhrel. Cross–sectional study of respiratory pathogens and their antibiotic susceptibility pattern in Tribhuvan University Teaching Hospital. Journal of Institute of Medicine Nepal. 2006 Aug31;28(2):5-9.doi:10.59779/jiomnepal.235.
27. Raghubanshi, B.R. & Karki, B.M.S. Bacteriology of Sputum Samples: A Descriptive Cross-sectional Study in a Tertiary Care Hospital. JNMA; journal of the Nepal Medical Association. 2020 Jan;58(221):24-doi:10.31729/ jnma.4807.
28. Sammodavardhana Kaundinnyayana, R.C., Anjan Khadka, Sabita Bhatta, Heleena Rayamajhi, Manoj Sharma. Antimicrobial Sensitivity among Bacterial Isolates from Sputum Samples in a Tertiary level Hospital. Journal of Institute of Medicine Nepal. 2024 04/30;46(1):25-30. doi:10.59779/Jiomnepal.1301.
29. Ahmed, T., Islam, T., Soha, R. & Akter, E. Correlation of the Pathogenic Bacteria Isolated from Sputum Samples with Age, Sex, Seasonal Variation and Determination of Their Antibiotic Resistance Pattern. Iranian Journal of Medical Microbiology. 2021 12/01;15:700-707. doi:10.30699/ijmm.15.6.700.
30. Panda, S., Nandini, B.P. & Ramani, T. Lower respiratory tract infection-bacteriological profile and antibiogram pattern. Int J Curr Res

Rev. 2012;4:149-55.
31. McGovern M, Kelly L, Finnegan R, McGrath R, Kelleher J., et al. Gender and sex hormone effects on neonatal innate immune function. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2024 Dec;37(1):2334850. eng. Epub 2024/06/06. doi:10.1080/14767058.2024.2334850.
32. Liu Y, Wang Y, Dong J, Wang J, Bao H., et al. Association between air pollution and emergency department visits for upper respiratory tract infection inLanzhou, China. Environmental science and pollution research international. 2022 Apr;29(19):28816-28828. eng. Epub 2022/01/07. doi:10.1007/s11356-021-17932-2.
33. Häder A, Köse-Vogel N, Schulz L, Mlynska L, Hornung F., et al. Respiratory Infections in the Aging Lung: Implications for Diagnosis, Therapy, and Prevention. Aging and disease. 2023 Aug 1;14(4):1091-1104. eng. Epu2023/05/10. doi:10.14336/ad.2023.0329.
34. Singh, S., Sharma, A. & Nag, V.L. Bacterial pathogens from lower respiratory tract infections: A study from Western Rajasthan. Journal of Family Medicine and Primary Care. 2020;9(3):1407-1412. doi:10.4103/jfmpc.jfmpc_994_19.
35. Koenen, M.H., de Steenhuijsen Piters, W.A.A., Bogaert, D. & Verhagen, L.M. The microbiota in respiratory tract infections: from association to intervention. Current opinion in infectious diseases. 2022 Jun 1;35(3):215-222. eng. Epub 2022/06/07. doi:10.1097/qco.0000000000000826.
36. De Steenhuijsen Piters, W.A., Sanders, E.A. & Bogaert, D. The role of the local microbial ecosystem in respiratory health and disease. Philosophical transactions of the Royal Society of London. Series B, Biological sciences. 2015 Aug 19;370(1675). eng. Epub 2015/07/08. doi:10.1098/rstb.2014.0294.
37. Kousalya K, Thirumurugu S, Arumainayagam DC, Manavalan R, Vasantha J., et al. Antimicrobial resistance of bacterial agents of the upper respiratory tract in South Indian population. Journal of advanced pharmaceutical technology & research. 2010 Apr;1(2):207-15. eng. Epub 2010/04/01.
38. Wang J-Y, Hsueh P-R, Wang J-T, Lee L-N, Yang P-C., et al. Recurrent Infections and Chronic Colonization by anEscherichia coli Clone in the Respiratory Tract of a Patient with Severe Cystic Bronchiectasis. Journal of Clinical Microbiology. 2000;38(7):2766-2767. doi:doi:10.1128/jcm.38.7.2766-2767.2000.
39. Martin-Loeches I, Reyes LF, Nseir S, Ranzani O, Povoa P., et al. European Network for ICU-Related Respiratory Infections (ENIRRIs): a multinational, prospective, cohort study of nosocomial LRTI. Intensive care medicine. 2023 Oct 9;49(10):1212-1222. doi:10.1007/s00134-023-07210-9.
40. Sherchan, J.B. & Humagain, S. Antimicrobial Susceptibility Pattern of Gram-Negative Bacteria Causing Lower Respiratory Tract Infections in Kathmandu University Hospital. Journal of Nepal Health Research Council. 2021Jan21;18(4):661-666. doi:10.33314/jnhrc.v18i4.2566.
41. Miriti, D.M., Muthini, J.M. & Nyamache, A.K. Study of bacterial respiratory infections and antimicrobial susceptibility profile among antibiotics naive outpatients visiting Meru teaching and referral hospital, Meru County, Kenya in 2018. BMC Microbiology. 2023 June 29;23(1):172. doi:10.1186/s12866-023-02905-x.
42. Choe, Y.J., Smit, M.A. & Mermel, L.A. Seasonality of respiratory viruses and bacterial pathogens. Antimicrobial resistance and infection control. 2019 Aug 2:125. doi:10.1186/s13756-019-0574-7.
43. Lambring, C.B., et al. Impact of the Microbiome on the Immune System. Critical reviews in immunology.2019;39(5):313-328. eng. doi:10.1615/CritRevImmunol.2019033233.
44. Yang, L., Liang, E. & Gao, Y. Modeling and simulation of distribution and drug resistance of major pathogens in patients with respiratory system infections. BMC infectious diseases. 2025 Jan 29;25(1):138. doi:10.1186/ s12879-025-10549-7.
45. Rincón, S., et al. [Resistance to “last resort” antibiotics in Gram-positive cocci: The post-vancomycin era]. Biomedica : revista del Instituto Nacional de Salud. Epub 2014 Jun

27. doi:10.1590/s0120-41572014000500022.
46. Ali, M., Mu'azu, L. & Abdallah, M. Isolation, Identification and Determination of Antibiotic Susceptibility Pattern of Some Bacteria Associated with Upper Respiratory Tract Infections. Archives of Pulmonology and Respiratory Medicine. 2020 Jan 1;3:10-16. doi:10.22259/2639-362X.0301002.
47. Goel, N., Chaudhary, U., Aggarwal, R. & Bala, K. Antibiotic sensitivity pattern of gram-negative bacilli isolated from the lower respiratory tract of ventilated patients in the Intensive care unit. Indian journal of critical care medicine : peer-reviewed, official publication of Indian Society of Critical Care Medicine. 2009 Dec 31;13(3):148-51. doi:10.4103/0972- 5229.58540
48. Adams, J., et al. Antimicrobial Stewardship Techniques for Critically Ill Patients with Pneumonia. Antibiotics (Basel, Switzerland). 2023 Feb 1;12(2). doi:10.3390/antibiotics12020295.
49. Yang L, Liang E, Gao Y. Global epidemiology of asymptomatic colonisation of methicillin- resistant Staphylococcus aureus in the upper respiratory tract of young children: a systematic review and meta-analysis. Archives of disease in childhood. 2024 Mar 19;109(4):267-274. doi:10.1136/archdischild-2023-326124.
50. Adane, F. & Esmael, A. Bacterial profile, antimicrobial susceptibility patterns, and associated factors among lower respiratory tract infection patients attending at Debre Markos comprehensive specialized hospital, Northwest, Ethiopia. BMC infectious diseases. 2025 Feb 24;25(1):266.
51. Nikaido, H. Multidrug resistance in bacteria. Annual review of biochemistry. 2009 Feb 24;78:119-46eng. doi:10.1146/.annurev.biochem.78.082907.145923