Multidrug-Resistant Bacteria with ESBL Genes: A Growing Threat Among HIV Patients in Nepal

Riju Maharjan
Tribhuvan University
Anup Bastola
Sukraraj Tropical and Infectious Disease Hospital
Nabaraj Adhikari
Tribhuvan University
Komal Raj Rijal
Tribhuvan University
Megha Raj Banjara
Tribhuvan University
Prakash Ghimire
Tribhuvan University
Upendra Thapa Shrestha (upendrats@gmail.com)
Tribhuvan University

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Abstract

Background

Bacterial opportunistic infections are quite common in HIV patients. Besides HIV-TB coinfection, lower respiratory tract infections by multidrug-resistant bacteria cause significant morbidity and mortality among HIV patients. This study was done to evaluate the bacterial coinfection of LRT and detect plasmid-mediated *bla*$_{TEM}$ and *bla*$_{CTX-M}$ genes among Extended-Spectrum β-Lactamase (ESBL) producing isolates from sputum samples in HIV patients.

Methods

A total of 263 sputum samples from HIV-positive cases were processed with standard microbiological methods to isolate and identify the possible pathogens. The identified bacterial isolates were assessed for antibiotic susceptibility pattern by using modified Kirby Bauer disk diffusion method following Clinical Laboratory Standard Institute (CLSI) guidelines. Plasmid DNA was extracted from multidrug-resistant and ESBL producers for screening of ESBL genes; *bla*$_{CTX-M}$ and *bla*$_{TEM}$ by conventional PCR method using specific primers.

Results

Of 263 sputum samples, 67 (25.48%) were culture positive showing *Klebsiella pneumoniae;* 17(25.37%) as the most predominant one. A higher rate of infection (4/8, 50%) was observed among old-aged people of 61 -70 years, whereas no infection was observed below 20 years. About 30.0% (15/50) of smokers, 32.86% (23/70) cases with previous pulmonary tuberculosis and 52.38% (11/21) with CD4 count <200 cells/μl were found to be susceptible to LRTIs. Among 53 bacterial isolates, 52.83% (n=28) were multidrug-resistant and 43.4% (n=23) were ESBL producers. All ESBL producers were sensitive to Colistin and Polymyxin B. Of 23 ESBL producers, 47.83% (11/23) and 8.6% (2/23) possessed only *bla*$_{CTX-M}$ and *bla*$_{TEM}$ genes respectively and 43.48% (10/23) possessed both ESBL genes.

Conclusion

The increasing rate of MDR bacterial infections mainly ESBL producers of LRTIs causes difficulty in the management of diseases leading to high morbidity and mortality of HIV patients.

Background

Human Immunodeficiency Virus (HIV), since first confirmed in 1981, has killed more than one million people worldwide. Thirty-eight million people were living with HIV at the end of 2019 with 1.8 million

people becoming newly infected (1). The virus destroys the function of immune cells like helper T cells specifically CD4+ T cells, macrophages and dendritic cells reducing the significant number of CD4 cell count (2) leading to an increased number of opportunistic infections. Although 67% of total HIV patients were covered with antiretroviral therapy (ART) globally, the secondary infections by fungi, bacteria, parasites and other viruses have not been reduced as expected. Bacterial and fungal infections are quite common opportunistic infections followed by parasitic and viral infections (3). The mortality rate of HIV patients is significantly higher in HIV and tuberculosis (TB) coinfection, however, the bacterial lower respiratory tract infections (LRTIs) other than by *Mycobacterium tuberculosis* can't be avoided in people living with HIV-AIDS (PLHA). *Streptococcus pneumoniae, Hemophilus influenza, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter* spp are some common organisms causing LRTIs (4). The antimicrobial resistance among those bacterial pathogens is a matter of concern nowadays due to the increase in the ineffectiveness of antibiotic therapy in PLHA leading to higher mortality (5).

In the context of Nepal, 10,000-15,000 people die of AIDS-related infections every year due to a lack of effective treatment and care (6). In most developing countries, the lack of proper diagnosis and treatment of secondary bacterial infection is a key to deteriorating the quality of life of PLHA. A very few studies have been documented on such infections and their impacts on PLHA in Nepal. Hence, this study was conducted to identify possible bacterial pathogens causing LRTI in PLHA and their antibiogram phenotypically and genotypically.

Methods

Study design, site and duration

A hospital-based prospective cross-sectional study was designed and was conducted in Antiretroviral Treatment (ART) center, Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal from February to August 2019. Sample collection and processing were done in the same hospital and the further process of genome extraction and gene detection was done in Central Department of Microbiology, Tribhuvan University, Kathmandu.

Inclusion and exclusion criteria

All age groups of both sexes living with HIV under ART who gave written consent were enrolled in this study. However, among them, if infected with tuberculosis were excluded from the study.

Sample size

The sample size was calculated to be 255 based on the prevalence rate of bacterial lower respiratory tract infection in HIV patients i.e. 21% as described by Kandati et al 2016 (7). For this study, a total of 263 sputum samples were collected during the study period.

Sample collection, transportation and processing

A sputum sample was collected from the confirmed HIV people visiting ART center at the hospital for ART. Sterile vials with a wide mouth and tight lid were provided to the participants for the collection of sputum. Participants were instructed to take a deep breath and then to expectorate cough (8). The sputum samples collected were transported to the Microbiology laboratory and processed. The quality of sputum was checked macroscopically for the presence of mucopurulent part for the acceptance of the sample.

Sputum culture

Mucopurulent sputum was cultured on MacConkey Agar (MA), Blood Agar (BA), and Chocolate Agar (along with 10-unit Bacitracin disk) and incubated at 37^0C for 24 hours. Incubated plates were examined for the presence of distinct colonies and identification was done by using standard microbiological protocols including their colony morphology on different culture media, microscopically by Gram staining and various biochemical tests (8).

For identification of *H. influenza* satellitism test was performed. A loopful of suspected colonies of *Haemophilus* colonies was mixed in about 2 ml of sterile physiological saline. Using a sterile swab stick, inoculate the organism suspension on a plate of blood agar and a pure culture of *S. aureus* was streaked across the inoculated blood agar plate. It was then incubated in a CO_2 enriched atmosphere at 35 to 37°C for 18-24 hours. The culture plate was examined for growth and satellite colonies (8).

Antibiotics susceptibility test

Antibiotic susceptibility patterns of the organism were performed by using the modified Kirby Bauer disk diffusion method recommended by CLSI 2019 (9). The antibiotics used were Amoxicillin (10 µg), Piperacillin/tazobactam (PIT, 100/10 mcg), Chloramphenicol (C, 30 mcg), Ciprofloxacin (CIP, 5 mcg, Gentamycin (GEN, 10 mcg), Tetracycline (30 mcg), Imipenem (IMP, 10 mcg), Amoxycillin/clavulanic acid (AMC, 20/10 mcg), Cefepime (CPM, 30mcg), Cotrimoxazole (COT, 25 mcg), Ceftazidime (CTZ, 30 mcg), Ceftriaxone (CTR, 30 mcg), Polymyxin-B (PB, 300 units) and Colistin (CL 10 mcg). The isolates that are resistant to at least 1 agent in ≥ 3 antimicrobial categories are considered as MDR (10). Subsequently, the prevalence of MDR bacteria was determined.

Screening and confirmation of extended-spectrum β-lactamase (ESBL)

Those isolates resistant to antibiotics; Ceftazidime (30µg), Ceftriaxone (30 µg) were considered as potential ESBL producers and the confirmatory test was performed by double disk diffusion method using Ceftazidime and Ceftazidime clavulanic acid. More than 5mm zone of diameter around Ceftazidime-clavulanic acid disc than Ceftazidime disc alone was confirmed to be an ESBL producer (9).

Detection of metallo β-lactamase (MBL)

Two Imipenem discs were placed on an MHA plate inoculated with a test organism (bacterial density equivalent to 0.5 Mac-farland Standard). A 5µl of EDTA (0.5M, pH=8.0) solution was added to one of the

Imipenem discs and incubated for 16-18 hours at 37^0C. An increased zone of diameter (>7mm) around Imipenem and EDTA disc than Imipenem alone was confirmed to be MBL positive (9).

Bacterial plasmid DNA extraction

Plasmid DNA from MDR isolates was extracted manually by phenol-chloroform method (alkaline lysis method) and visualized by running Agarose gel electrophoresis as described by Sambrook, 1989 and Thapa Shrestha and Adhikari 2014 (11, 12).

Molecular detection of bla_{CTX-M} **and** bla_{TEM} **gene**

A set of primers for each gene had been selected from the previous studies and verified on NCBI BLAST. A set of primers (Forward: 5′-TTTGCGATGTGCAGTACCAGTAA-3′ and reverse: 5′-CTCCGCCTGCCGGTTTTAT-3′) as described in Edelsteint et al 2003 (13) were used for bla_{CTX-M} gene. Similarly, for the $bla_{TEM,}$ a set of primers (Forward: 5′-GAGACAATAAGGGTGGTAAAT-3′ and reverse: 5′- AGAAGTAAGTTGGCAGCAGTG-3′) as mentioned in Sharma et al. 2013 were used (14). A conventional PCR was used to amplify the bla_{TEM} and bla_{CTX-M} genes. A PCR reaction mixture was prepared to contain 12.5 µl the master mix from Qiagen, 0.5 µl of each primer, 3 µl template DNA and 8.5 µl PCR grade water (13). PCR Amplification reactions were run at initial denaturation of 95^0C for 15 minutes; followed by 35 cycles of denaturation at 94^0C for 45 seconds, annealing at 55^0C for bla_{TEM} genes and 56^0C for the bla_{CTX-M} gene for 30 s, extension at 72^0C for 3 min and a final extension at 72^0C for 10 min. The PCR products were analyzed on 1.5% agarose gel electrophoresis with 0.2 µg/ml concentration of ethidium bromide.

Quality Control

Control strain of *E. coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were used as positive controls from ESBL and MBL producing strains respectively. For PCR, a previously harvested plasmid containing target genes were used as positive control and nuclease-free water as a negative control.

Data management and analysis

All the data collected from the laboratory about the isolates and demographic data collected from the questionnaire were processed and analyzed by using SPSS-21 software. The significance of the data with different variables was calculated by the Chi-square test. P-value < 0.05 was considered as a significant result.

Results

Mode of transmission of HIV among study population: Among 263 cases, 145 (55.13%) were male and 118 (44.87%) were female. Heterosexual activities were the most dominant route of transmission accounting for 78.33% whereas the least rate of transmission, 0.76% (n=2) was observed via a vertical route from mother to infants (Figure 1).

Bacterial growth according to age and gender of patients

Out of 263 sputum samples from HIV-positive individuals, 67 (25.48%) samples were positive for bacterial growth. The bacterial infection of LRT was found highest (50%) in the age group 61-70 years followed by 40% (n=10) among the male of age group 51-60 years. No infection was observed below 20 years (Table 1).

Table 1
Age and sex-wise distribution of Microbial growth

Age group (years)	Male		Female		Total number (growth %)
	No. of sample	Growth (%)	No. of sample	Growth (%)	
Below 20	3	0	1	0	4 (0)
21-30	29	8 (27.59)	20	5 (25)	49 (26.53)
31-40	40	9 (22.5)	52	12 (23.08)	92 (22.83)
41-50	42	10 (23.81)	32	10 (31.25)	74 (27.03)
51-60	25	10 (40)	11	1 (9.09)	36 (30.56)
61-70	6	3 (50)	2	1 (50)	8 (50)
Total	145	40 (27.59)	118	27 (22.88)	263 (25.48)

Bacterial co-infection and predisposing factors: Bacterial infection was observed significantly higher (p=0.02) among HIV patients with CD4 count <200 cells/µl. Similarly, bacterial infections were found to be higher among smokers and those with a history of pulmonary tuberculosis but the data was not statistically significant (Table 2).

Table 2
Relation bacterial infection with predisposing factors

Predisposing factors	Status of predisposing factors	Bacterial Growth		Total	p-value
		Negative (%)	Positive (%)		
CD4 count cells/µl	< 200	10 (47.62)	11 (52.38)	21	0.02
	200-499	56 (60.22)	37 (39.78)	93	
	>500	130 (87.25)	19 (12.75)	149	
Total		**196 (74.52)**	**67 (25.48)**	**263**	
Smoking habit	Non-smoker	117 (77.48)	34 (22.52)	151	> 0.05
	Smoker	35 (70)	15 (30.0)	50	
	Previous smoker	44 (70.97)	18 (29.03)	62	
Total		**196 (74.52)**	**67 (25.48)**	**263**	
History of PTB	No cases	149 (77.20)	44 (22.80)	193	> 0.05
	Cases	47 (67.14)	23 (32.86)	70	
Total		196 (74.52)	67 (25.48)	263	

Bacterial infection among HIV people based on their literacy and occupation

Most of the samples (43.3%) were from HIV people completing primary level education. Very least (1.1%) samples were from highly educated ones. The rate of infection was found to be higher among literate people as compared to illiterate people. Likewise, bacterial infection was higher (30%) in the individuals involving their business followed by official workers (26.6%) (Table 3).

Table 3
Microbial growth pattern and literacy

Literacy	Number of sample	Bacterial growth (%)	Occupation	Number of sample	Bacterial growth (%)
Illiterate	85	26 (30.59)	Households	82	20 (24.39)
Primary	114	28 (24.56)	Farmer	36	10 (27.78)
Secondary	52	8 (15.38)	Business	50	15 (30)
Higher secondary	9	3 (33.33)	Driving	13	2 (15.38)
Bachelors	3	2 (66.67)	Social work	10	2 (20)
Total	**263**	**67 (25.48)**	Labor	9	2 (22.22)
			Official work	38	10 (26.32)
			Others	25	6 (24)
			Total	263	67 (25.48)

Frequency of bacterial pathogens from LRT infections: Out of 67-gram negative bacterial isolates, *K. pneumoniae* was the most predominant bacteria followed by *H. influenzae*. *Acinetobacter* spp was the least isolated one (Figure 2).

Antibiotic susceptibility pattern: All isolated strains of *K. pneumoniae* and *K. oxytoca* were resistant to Amoxicillin whereas all of them were sensitive to Colistin and Polymyxin B. Of 12 isolates of *E. coli*, 10 (83.33%) were resistant to Amoxicillin and 9 (75%) were resistant to third-generation cephalosporins. Similarly, the highest number of *E. aerogenes*; 8/9 (88.89%) were resistant to Amoxicillin. All isolates of *C. frundii* were also resistant to Amoxicillin (Table 4).

Table 4
Antibiogram of bacterial isolates of *Enterobacteriaceae* family

Antibiotics used	Number of resistant pathogens (%)				
	K. pneumoniae (n=17)	K. oxytoca (n=4)	E. coli (n=12)	E. aerogenes (n=9)	C. frundii (n=6)
Amoxicillin	17 (100)	4 (100)	10 (83.33)	8 (88.89)	6 (100)
Cotrimoxazole	10 (58.82)	2 (50)	8 (66.67)	1 (11.11)	0
Ceftriaxone	9 (52.94)	2 (50)	9 (75)	1 (11.11)	0
Ciprofloxacin	4 (23.53)	1 (25)	7 (58.33)	1 (11.11)	0
Chloramphenicol	3 (17.65)	0	3 (25)	1 (11.11)	0
Gentamycin	2 (11.76)	0	1 (8.33)	1 (11.11)	0
Tetracycline	14 (82.35)	3 (75)	7 (58.33)	3 (33.33)	3 (50)
Ceftazidime	9 (52.94)	2 (50)	9 (75)	1 (11.11)	0
Amoxicillin-clavulanic acid	10 (58.82)	2 (50)	9 (75)	2 (22.22)	0
Piperacillin tazobactam	5 (29.41)	1 (25)	7 (58.33)	1 (11.11)	0
Cefepime	5 (29.41)	1 (25)	5 (41.67)	1 (11.11)	0
Imipenem	2 (11.76)	0	1 (8.33)	1 (11.11)	0
Polymyxin B	0	0	0	0	0
Colistin	0	0	0	0	0

All strains of *P. aeruginosa* were resistant to Amoxicillin, Cotrimoxazole, Ceftriaxone, Ceftazidime, Chloramphenicol, Tetracycline, Amoxicillin-clavulanic acid, Piperacillin-tazobactam and 3/4 (75%) of strains were resistant to Ofloxacin and Cefepime. However, all strains were sensitive to Gentamycin, Ciprofloxacin, Imipenem, Amikacin, Colistin. Likewise, all isolates of *Acinetobacter* spp were resistant to Amoxicillin, Cotrimoxazole, Ceftriaxone, Chloramphenicol, Tetracycline, Ceftazidime, Piperacillin and Amoxicillin-clavulanic acid and all were susceptible to Gentamycin, Imipenem, Polymyxin B and Colistin.

Multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL) producing strains

Out of 53 bacterial isolates, 28 (52.83%) were found multidrug-resistant. All strains of *P. aeruginosa* and *Acinetobacter* spp were found MDR strains whereas no strain of *C. frundii* was observed to be MDR. Similarly, 75% (9/12) of *E. coli*, 64.71% (11/17) of *K. pneumoniae* and 50% (2/4) of *K. oxytoca* were also MDR strains.

A total of 23 (43.4%) isolates were found to be ESBL producers including 75% (9/12) *E. coli* and all isolates of *P. aeruginosa* and *Acinetobacter* spp.

Out of total MDR strains, 72.73% (8/11) of *K. pneumoniae* and 50% (1/2) of *K. oxytoca* were ESBL producers. One strain of *P. aeruginosa* was also found to produce MBL.

Detection of ESBL genes: Of 53 isolates, 23 (43.4%) possessed ESBL genes. Among them, 11 (47.83%) harbored *the* $bla_{CTX\text{-}M}$ gene (Figure 3) and 2 (8.69%) contained *the* bla_{TEM} gene only (Figure 4). Similarly, 10 isolates (43.48%) possess both bla_{TEM} and $bla_{CTX\text{-}M}$ genes in their plasmid DNA (Table 5).

Table 5
Detection of bla_{CTX-M} **and** bla_{TEM} **ESBL genes among the isolates**

Isolates	n	Phenotypic ESBL	Genotypic ESBL	Number of amplified genes		
				bla_{CTX-M} (%)	bla_{TEM} (%)	bla_{CTX-M} + bla_{TEM} (%)
K. pneumoniae	17	8 (47.06)	7 (41.18)	3 (42.86)	0	4 (57.14)
K. oxytoca	4	1 (25)	2 (50)	1 (50)	0	1 (50)
E. coli	12	9 (75)	9 (75)	3 (33.33)	2 (22.22)	4 (44.45)
P. aeruginosa	4	4 (100)	3 (75)	3 (100)	0	0
Acinetobacter spp	1	1 (100)	1 (100)	1 (100)	0	0
E. aerogenes	9	0	1 (11.11)	0	0	1 (100)
C. frundii	6	0	0	0	0	0
Total	53	23 (43.4)	23 (43.4)	11 (47.83)	2 (8.69)	10 (43.48)

Discussion

Among the different routes of HIV transmission, most of the people in this study were infected through the heterosexual route (78.33%). Similar to this study, Chandwani et al reported the highest rate (95%) of transmission via the heterosexual route (15). Among the infected people, almost all females were housewives and got HIV infections from their husbands. This result is also supported by Chandwani et al (15). The least rate of transmission from mother to infants might be due to an increase in awareness on HIV-AIDS and wide coverage by ART reducing the rate of transmission as recommended by WHO (1).

Of 263 samples processed, 67 (25.5%) were found culture positive. Similar studies by Chakma et al and Ojha reported higher rates of bacterial infection among HIV people accounting for 78.3% and 46.6% of respectively (4, 16). Likewise, Oja-Bola and Oluyege reported 55.6% of HIV people associated with pneumonia (5) and 52.83% by Ramana et al (17). The comparatively lower rate of bacterial infections might be due to the Cotrimoxazole prophylaxis which is recommended for people under ART by WHO (18). In addition, HIV people having lower CD4 cells were given IPT (Isoniazid preventive treatment) to prevent MTB. IPT completed people may reduce the rate of isolation of the bacterial pathogen from LRT (17). A relatively higher occurrence of LRT infection was observed in the old age of 61-70 years as compared to other groups (p-value 0.49). The weakness of the immune system with age makes them vulnerable to different types of infections. Macfarlane et al 1993 also reported the same (19). No infection was reported in young participants of age below 20. The infection rate was higher in male participants (27.5%) as compared to female participants (22.88%). Similarly, Ojha et al reported the rate of infection in males and females in the ratio of 1.3:1 (p=0.39) (4).

Many predisposing factors are associated with bacterial coinfection in HIV people. A significantly higher rate of bacterial infection (39.8%) was observed in HIV patients having CD4 count <200 cells/µl as compared to the least infection in PLHA with CD4 count >500 cells/µl. Yadav and Prakash also observed a significantly higher rate of LRT infections (63.4%) among cases with a CD4 count <200 cells/µl, followed by those within the 200-500 range category (53.1%) and least (18.7%) in that above 500 cells/µl (20). The most important risk factor for bacterial pneumonia in HIV people is the degree of immunosuppression which is reflected by the CD4+ T-lymphocyte count (21). On the other hand, a slightly higher rate of bacterial infection was observed in active smokers (p=0.43). In contrast, Yadav and Prakash reported a significantly higher rate of LRT infection among smokers (20). They also concluded that tobacco smoking has been identified as one of the most important risk factors contributing to a higher prevalence of chronic bronchitis and chronic obstructive lung disease in Nepal. Various mechanisms cause increasing susceptibility of smokers to various infections including structural changes in the respiratory tract and decreased immune response (22). Thirdly, a bacterial infection in HIV people previously infected with PTB was comparatively higher than those without a history of PTB (22.8%) (p=0.06). Since PTB is a chronic lung disease and leaves patches, pulmonary nodules, or granuloma in the lungs, which might enhance the secondary bacterial infection (23). Likewise, people who were more exposed to the external environment such as shopkeepers, carpenters, tourist guides, security guards, army, police, etc were found more susceptible to LRT infections as compared to other people doing household. The reason might be due to exposure to the polluted outdoor environment.

Among 67 Gram-negative isolates, *K. pneumoniae* was the predominant one followed by *H. influenzae* and *E. coli*. Similar to this study, Ramana et al. and Jemal et al. found *K. pneumoniae* as a predominant one accounting for 45.1% and 41.3% respectively (17, 24). Few other studies had also reported *K. pneumoniae* as the predominant bacteria causing LRTI among HIV people (4, 25). However, Oja-Bola and Oluyege reported *E. coli* (40%) as the most frequent organism followed by *P. aeruginosa* (35%) and *K. pneumoniae* as the least (5%) isolated one (5). *K. pneumoniae* can cause diseases in non-HIV people as well however pneumonia due to *Klebsiella* is classically thought to be community-acquired (25). However,

Mishra and the coworkers reported *Haemophilus influenza* as the most predominant (21%) isolate in the sputum of HIV people (26). *H. influenzae* associated pneumonia is highly associated with a high degree of immune suppression (27).

We found Amoxicillin, Tetracycline and Cotrimoxazole were the least effective drugs against Gram-negative pathogens, however, WHO recommended Cotrimoxazole for the treatment of pneumococcal disease in HIV/AIDS patients. This result was also supported by Oja-Bola and Oluyege (5). They found only 20% of *Klebsiella* that were sensitive towards Cotrimoxazole. Similarly, Adeleye et al. reported Cotrimoxazole to be resistant for LRT isolates in Nigeria (28). The resistance towards trimethoprim-sulphamethoxazole (TMP/SMX) might be due to the extensive use of Cotrimoxazole by HIV-infected people as their basic regimen for the treatment of opportunistic bacterial infections. In the study, ESBL producing strains were found to be resistant towards most of the drugs used except Gentamycin, Imipenem, Polymyxin B and Colistin. And non-ESBL producing isolates were sensitive towards all drugs except Amoxicillin and Tetracycline.

Plasmid-mediated ESBL producers are nowadays a matter of concern due to their capacity to hydrolyze 3rd and 4th generation cephalosporin and monobactams (29). Decreased susceptibility of Gram-negative isolates towards 3rd and 4th generation cephalosporin could be attributed to ESBL or AMP-C beta-lactamase production. The most predominant ESBL producer was found to be *E. coli, P. aeruginosa* and *Acinetobacter* spp followed by *K. pneumoniae* by the phenotypic method. In contrast to our result, KC et al had reported *K. pneumoniae* (80%) and *P. aeruginosa* (50%) as the two predominant species to produce ESBL from the same study site (25).

On amplifying the plasmid-mediated ESBL gene, 23 (43.3%) isolates gave a positive result. The two isolates that gave positive ESBL test phenotypically contained neither of the two genes amplified. It might be due to the presence of ESBL genes other than targeted ones. This result was supported by de Oliveira et al (30). Another two isolates were possessing ESBL genes but were phenotypically undetectable by combined disk test. This showed the sensitivity of the genotypic method over the phenotypic method. Another reason might be a low level of ESBL gene expression. Gautam et al also reported 40.8% of PCR-positive ESBL that were phenotypically undetectable (31). They also explained that phenotypic identification of ESBL was based on the inhibition of the enzyme by clavulanic acid, and the inhibitory action of clavulanic acid could be masked due to the co-existence of multiple enzymes. In addition, the co-existence of AmpC enzymes in ESBL producers may alter the pores of the cell membranes, resulting in reduced affinity for β-lactamase inhibitors for enzymes such as TEM and SHV. Hence, the production of different types of β-lactamases (TEM, SHV, CTX-M and OXA) by the same microorganism can lead to erroneous phenotypic conclusions.

Study limitation

All possible ESBL genes were not detected due to lack of time and budget.

Conclusion

The lower respiratory tract infections other than PTB by multidrug-resistant Gram-negative pathogens are quite common opportunistic infections deteriorating the quality of life of HIV people. The higher rate of resistance towards WHO recommended broad-spectrum antibiotic, Cotrimoxazole and the presence of drug-resistant genes alarms the rampant use of antibiotics among seropositive HIV individuals.

Abbreviations

AIDS: Acquired Immune Deficiency Syndrome, ATCC: American Type Culture Collection; ART: Antiretroviral Treatment, AST: Antibiotic Susceptibility testing; BA: Blood Agar; CA: Chocolate Agar, CDC: Center for Diseases Control and Prevention, CLSI: Clinical Laboratory Standard Institute; DOHS: Department Of Health Service; ESBL: Extended Spectrum b-Lactamase; HIV: Human Immunodeficiency Virus, IPT: Isoniazid Preventive Treatment, LRTI: Lower Respiratory Tract Infection, MA: Mac-Conkey Agar, MBL: Metallo b-Lactamase; MHA: Mueller Hinton Agar; MDR: Multidrug Resistance; min: minute/s, PCR: Polymerase chain reaction, PLHA: People Living with HIV-AIDS, PTB: Pulmonary Tuberculosis, STIDHH: Sukraraj Tropical and Infectious Disease Hospital, WHO: World Health Organization

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by Nepal Health Research Council (NHRC). A copy of the information sheet and a consent form were given to the participants to obtain written consent before enrollment in the research and collecting samples. A local language translated information sheet was read for illiterate participants. Informed consent was obtained from all the participants and in the case of children under 16 years, both written informed consent with assent was obtained from a parent or a local guardian attending the hospital along with the participant. All the methods were carried out in accordance with the principles stated in the Declaration of Helsinki.

Consent to publish

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request at upendrats@gmail.com.

Competing interests

We declare no competing interests.

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Authors' Information:

RM Riju Maharjan, M.Sc. Email: rjunewa50@gmail.com

AB Anup Bastola, MD Email: docanup11@gmail.com

NA Nabaraj Adhikari, Ph.D.Email: adhikarinaba13@gmail.com

KRR Komal Raj Rijal, Ph.D. Email: rijalkomal@gmail.com

MRB Megha Raj Banjara Email: banjaramr@gmail.com

PG Prof. Prakash Ghimire Email: prakashghimire@gmail.com

UTS Upendra Thapa Shrestha, Ph.D. Email: upendrats@gmail.com

Authors' contributions

RM developed the concept of this study and the whole laboratory work was done by herself. The HIV people were convinced and enrolled in the study by AB and also supervised the hospital work at STIDH. The molecular work was supervised UTS at Central Department of Microbiology, Tribhuvan University. The data were analyzed by RM, UTS and MRB. The first draft of the manuscript was prepared by UTS. The review of the manuscript was done by NA, KRR and PG. The final draft was prepared by UTS. All the authors have read and reviewed the manuscript thoroughly.

Authors' Details

[1]Central Department of Microbiology, Tribhuvan University, Kirtipur, Kathmandu, Nepal

[2]Sukraraj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal

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Figures

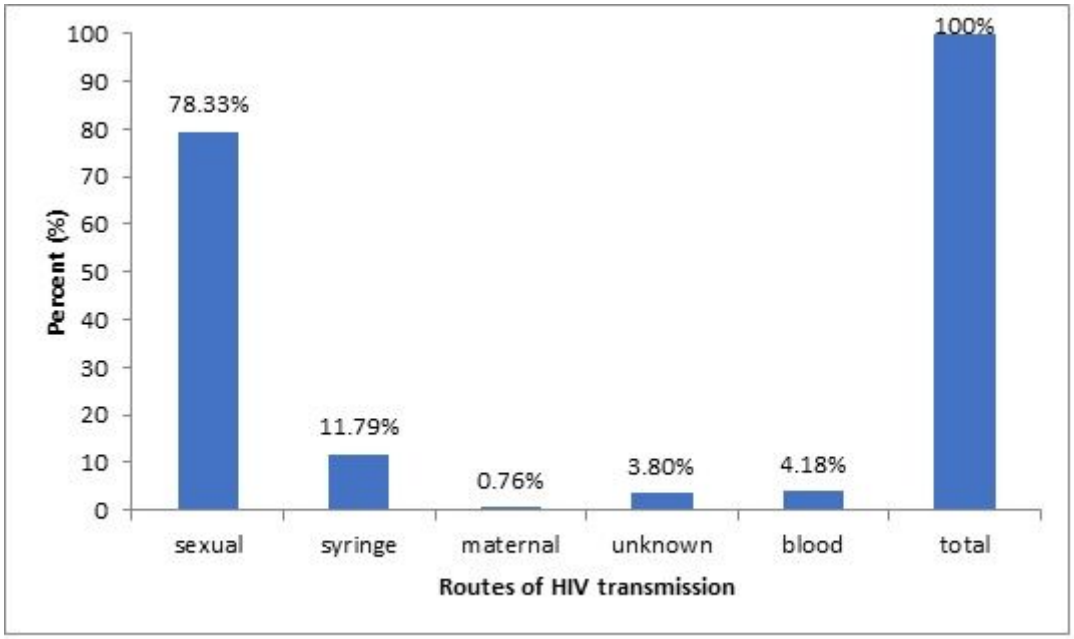

Figure 1

Mode of transmission of HIV among participants

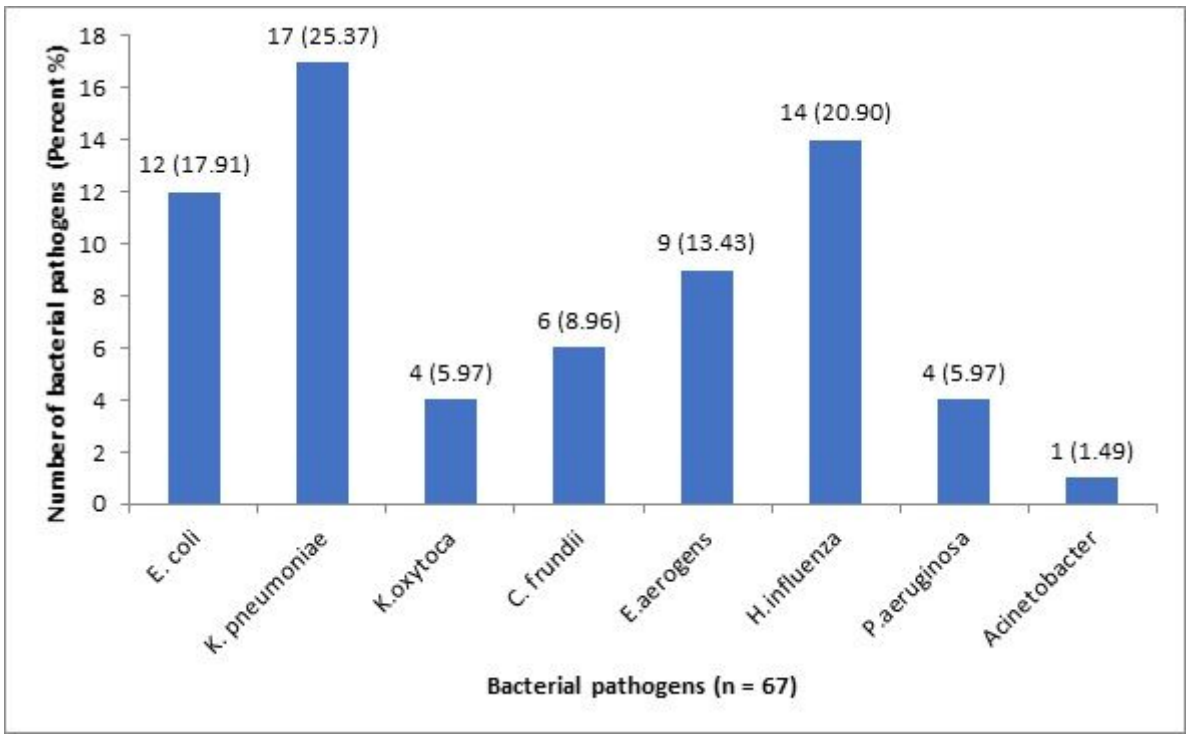

Figure 2

Distribution of bacterial pathogens in LRT infections of HIV people

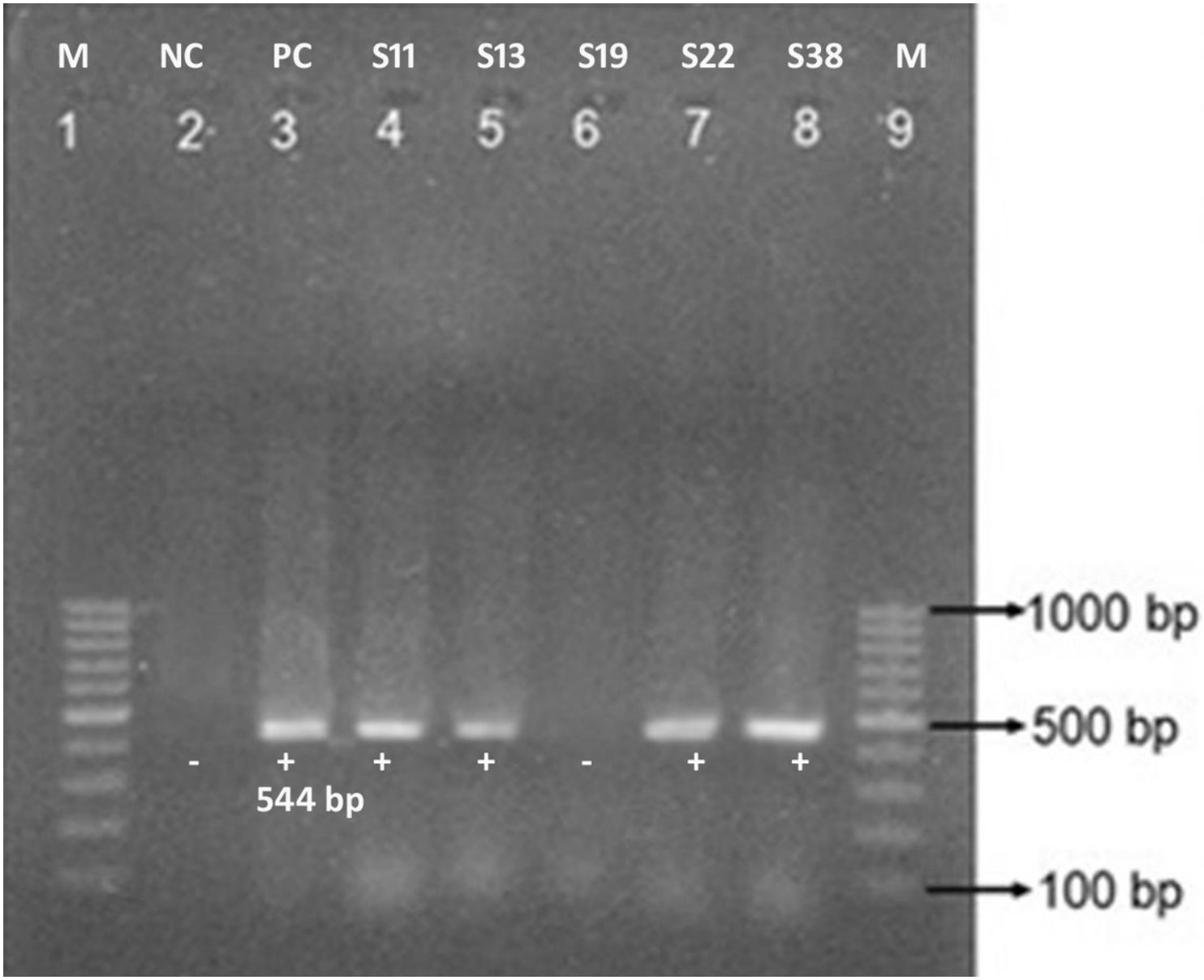

Figure 3

Amplification of *bla*$_{CTM}$ gene (Lane 1 and 9; DNA marker (100 bp), Lane 2; NC (Negative control), Lane 3; PC (Positive control) and Lane 4-8; gene amplification from isolates)

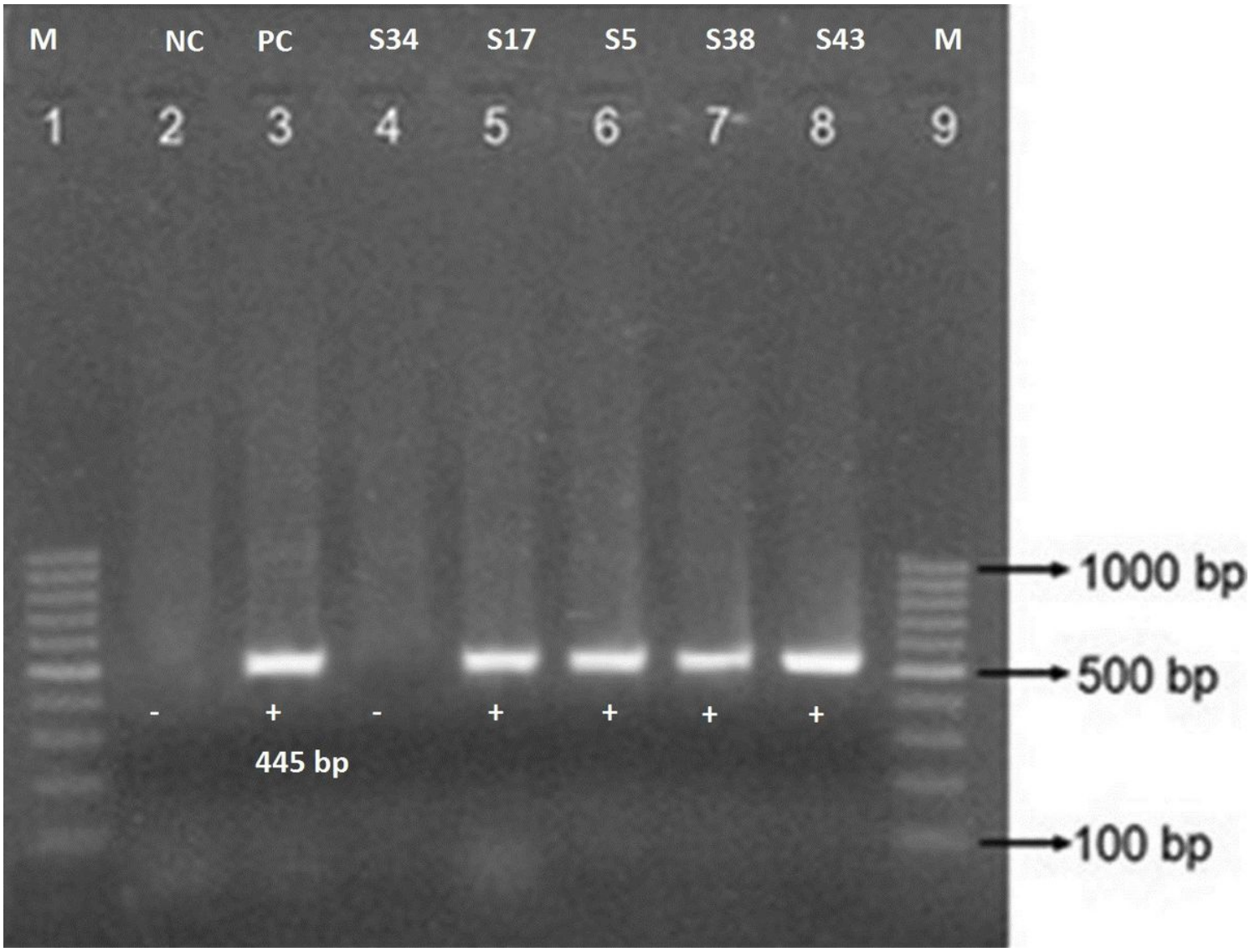

Figure 4

Amplification of *bla*$_{TEM}$ gene (Lane 1 and 9; DNA marker (100 bp), Lane 2; NC (Negative control), Lane 3; PC (Positive control) and Lane 4-8; gene amplification from isolates)