ANTIBIOTIC RESISTANCE PATTERN OF BACTERIA ISOLATED FROM CLINICAL SPECIMENS: A HOSPITAL-BASED CROSS-SECTIONAL STUDY IN KATHMANDU, NEPAL

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ABSTRACT

Antibiotics are vital in combating infectious diseases, yet their increasing use fosters resistance. Antimicrobial resistance (AMR) is rising in Nepal due to factors such as indiscriminate, inappropriate, and inadequate antibiotic usage. This study aims to explore the association between demographic factors and the prevalence of specific bacterial strains within the surveyed population. Additionally, it seeks to determine the antibiotic resistance patterns of these bacteria. Antibiotic susceptibility or resistance data were retrieved from the Medical Records Department (MRD) of the Manmohan Memorial Medical College and Teaching Hospital (MMMCTH) in Kathmandu. Samples from patients with certain types of bacterial infections were included, with 56 from sputum reports, 46 from urine, and 8 from blood samples out of 110 retrieved. Analysis revealed that sputum samples were mostly from older males, while urine samples were mostly from females. Yet, gender did not significantly influence bacterial presence across sample types. Overall, Escherichia coli was the most prevalent bacterium (74%), followed by Salmonella typhi (25%), Staphylococcus aureus (25%), and Klebsiella pneumoniae (23%) isolated from different type of clinical samples. Altogether, 6-15 antibiotics were assessed for sensitivity, with 2–6 antibiotics showing sensitivity to blood bacteria, 1-6 antibiotics demonstrating sensitivity to sputum bacteria, and 3–8 antibiotics exhibiting sensitivity to urine bacteria. Many investigated antibiotics were resistant, only gentamicin exhibited sensitivity for all types of bacteria found in blood, sputum and urine. These findings underscore the importance of discerning bacterial resistance patterns for effective antimicrobial treatment selection.

KEYWORDS

Antimicrobial resistance, clinical specimens, bacterial isolates, Nepal

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INTRODUCTION

Antibiotics are essential, life-saving medications used to treat bacterial, fungal, and protozoal infections, combating infectious diseases. Antimicrobial resistance (AMR) stands as a paramount global public health and development threat. It is estimated that bacterial AMR directly accounted for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths.[1] Extensive use of antibiotics, leading to AMR, is increasing globally.[2] In Nepal, AMR is increasing mainly due to the indiscriminate, inappropriate and insufficient use of antibiotics.[3–5] In addition, high rates of infectious disease, the use of antibiotics as a growth promoter, and the poor implementation of AMR legislation are suggested as main contributors of AMR in underdeveloped nations.[6]

Several factors are being suggested for emergence of antimicrobial resistance. In addition to human infection and treatment factors, increased demand of animal food is also suggested as contributor of AMR. Moreover, infection prevention practice, lack of proper surveillance, living conditions, access to sanitation infrastructure, population growth and urbanization, and gaps in policy implementation have been suggested as contributors of AMR.[7] Environmental factors are also suggested to contribute to antimicrobial resistance, including soil related factors, waste management, potable and wastewater mainly from the Asian region in aiding infection burden.[8] For example, the burden of infectious diseases such as respiratory tract infection (RTI), enteric fever, urinary tract infection (UTI) and other bacterial infections is exceedingly high in Nepal as it is the majority of low and lower middle income countries (LMICs).[9] Specifically, research findings from LMICs show that poor prescription practices, along with attitudes and knowledge regarding antibiotics, contribute to the rise in AMR.[10]

In an underdeveloped country like Nepal, antibiotics are over-prescribed and easily brought from any pharmacy without a doctor's prescription.[11] Elements that contribute to the excessive use of antibiotics in underdeveloped countries include insufficient patient education, a lack of suitable diagnostic resources, the availability of antimicrobials that can be obtained over-the-counter, and a lack of effective drug regulatory processes.[12] The over use of antibiotics has led to unnecessary antibiotic exposure, putting patients at risk for major ill effect[13] as antibiotics lost their effectiveness against common bacterial infections due to regular use. Therefore, AMR particularly in Nepal is rising before it is acknowledged as a serious issue.[2] Besides excessive use of antibiotic, other factor contributing to the growing burden of AMR is the illogical prescription of even newer generation antibiotics or last resort antibiotics.[5] Though the issue can be resolved with a limited spectrum of medications, some doctors still recommend broad-range antibiotics.[5]

A well-known strategy to maintain the potency of current medications is to create and adhere to guidelines and practices for prescribing antibiotics. However, the majority of hospitals in Nepal lack appropriate policies and procedures for the administration of antibiotics, or they may not fully comply with the national guidelines.[13] Despite ample epidemiological evidence regarding AMR,[4] data on socio-demographic and socio-economic factors associated with AMR are still scarce in Nepal across different populations. Hence, our study aims to explore the prevalence of AMR and the association of socio-demographic factors with the prevalence of specific strains of bacteria in the surveyed population using hospital laboratory records.

MATERIALS AND METHODS

Study area: This study was conducted at Manmohan Memorial Medical College and Teaching Hospital (MMMCTH) in Kathmandu, Nepal established in 2013. MMMCTH is a tertiary care hospital which provides 24-hour emergency services and has a capacity of 300 beds with well-equipped facilities. Our sample was considered widely representative as the hospital claims to contribute to the national health system by delivering quality care to underprivileged and marginalized populations at an affordable cost, and by fostering the development of quality health human resources as required by the country. This hospital was established in honour of the ex-Prime Minister of Nepal, Mr. Man Mohan Adhikari, who was a leader of the underprivileged class.

Data collection: A hospital-based cross-sectional survey was conducted during the month of March 2023 to assess AMR. Patients tested growth positive for bacterial infections were included as the sample population for the study. A total of 110 patients with bacterial infections were considered for further analysis. Data related to susceptibility and resistance to specific types of bacteria were retrieved from the hospital's Medical Records Department (MRD), including demographic information (age, sex) of the patients, while maintaining confidentiality. AST was done by Kirby-Bauer disc diffusion method following CLSI guidelines.

Of the 110 samples, 49 were from male and 61 were from female.

Data analysis: The secondary data were sorted and coded in MS Excel. Descriptive and inferential statistics were then employed for data analysis. The level of significance was set at p <0.05. All statistical analyses were conducted using SPSS-20.

RESULTS

Table-1 illustrates that the study participants were mostly middle-aged females, with a mean age of 53.17 years. Out of the 110 samples with sensitivity reports retrieved, sputum samples predominated (n=56), followed by urine (n=46) and blood samples (n=8). Sputum samples were mostly from males (65.0%), while

Table 1: Characteristic features of study participants (n = 110)

Characteristics	Male (n=49) Mean (SD)/n (%)	Female (n=61) Mean (SD)/n (%)	P-value	Total Mean (SD)/n (%)
Demographic characteristics				
Age (in year)	53.70 (22.6)	52.77 (19.0)	NS$	53.17 (20.9)
Total sensitivity %	62.42 (20.7)	59.25 (22.7)	NS$	60.66 (21.8)
Types of samples				
Blood	4 (8.2)	4 (6.6)	0.01#	8 (7.3)
Sputum	32 (65.3)	24 (39.3)		56 (50.9)
Urine	13 (26.5)	33 (54.1)		46 (41.8)

$: Independent T-test, # Fisher exact test

Table 2: Demographic characteristics and prevalence of bacteria and sensitivity results (n = 110)

Characteristics	Male (n=49) Mean (SD)/n (%)	Female (n=61) Mean (SD)/n (%)	P-value	Whole Population Mean (SD)/n (%)
Blood sensitivity test parameter				
Age (in year)	36.00 (0.0)	31.00 (6.8)	NS$	32.00 (6.3)
Total sensitivity %	68.33 (23.3)	69.04 (19.1)	NS$	68.69 (19.8)
Blood bacteria (n=8)	N=4	N=4		
Kleibsella pneumoniae	0 (0.0)	2 (50.0)	NS#	2 (25.0)
Staphylococcus aureus	1 (25.0)	1 (25.0)		2 (25.0)
Salmonella typhi	0 (0.0)	1 (25.0)		1 (12.5)
Sputum sensitivity test parameter	N=32	N=24		
Age (in year)	54.16 (21.6)	64.54 (16.)	**0.045**$	58.61 (20.0)
Total sensitivity %	62.81 (21.5)	48.62 (26.73)	**0.032**$	56.73 (24.7)
Sputum bacteria (n=56)				
Kleibsella pneumoniae	8 (25.0)	5 (21.0)	NS#	13 (23.0)
Enterobacter aerogenes	4 (13.0)	3 (13.0)		7 (13.0)
ACBC II	2 (6.0)	4 (17.0)		6 (11.0)
Pseudomonas aeruginosa	7 (22.0)	4 (17.0)		11 (20.0)
Urine sensitivity test parameter	N=13	N=33		
Age (in year)	53.92 (26.3)	46.66 (18.3)	NS$	48.76 (20.8)
Total sensitivity %	59.65 (19.2)	65.80 (16.8)	NS$	64.06 (17.5)
Urine bacteria				
Escherichia coli	8 (62.0)	26 (79.0)	NS#	34 (74.0)
Proteus vulgaris	2 (15.0)	0 (0.0)		2 (4.0)
Staphylococcus aureus	0 (0.0)	2 (6.0)		2 (4.0)
Salmonella typhi	0 (0.0)	2 (6.0)		2 (4.0)

$: Independent T-test, # Fisher exact test, ACBC II =Acinetobacter calcoaceticus-baumannii complex the majority of urine sample reports were from female participants (54.1%). The age of participants overall, or for each specimen type, was comparable between male and female participants, except for sputum samples.

Table-2 presents demographic characteristics and the prevalence of bacteria, along with sensitivity results. Sputum samples were predominantly from older females, with lower sensitivity observed among females compared to males. Notably, none of the bacteria in all three types of samples showed significant differences between male and female participants. In terms of bacterial frequency, *Escherichia coli* was the most prevalent bacteria, with a prevalence rate of 74% (34/46 urine samples cultured), followed by *Klebsiella pneumoniae* and *Staphylococcus aureus*, each with a prevalence of 25% (2/8) blood samples. *K. pneumoniae* also had a prevalence of 23% (13/56) sputum samples. The highest number of bacteria was observed in sputum samples, followed by urine and blood, according to sample size.

Among the different antibiotics assayed for sensitivity, gentamicin was most effective (100.0%) for all types of bacteria in blood samples. Similarly, gentamicin was effective also for sputum and urine bacteria (82.0%). On average, 6-15 antibiotics were evaluated for blood, urine, and sputum culture and sensitivity testing. Among these, 2-6 antibiotics were found to be effective for blood bacteria, 1-6 antibiotics for sputum bacteria, and 3-8 antibiotics for urine bacteria. Out of the 6-15 antibiotics evaluated for blood, urine, and sputum isolates, 1-8 antibiotics were found to be not effective for blood bacteria, 1-12 antibiotics for sputum bacteria, and 1-8 antibiotics for urine bacteria.

In terms of bacterial frequency, *E. coli* was the most prevalent bacteria, with a prevalence of 74.0% (34 out of 46 urine samples cultured), followed by *K. pneumoniae* and *S. aureus*, each with a prevalence of 25.0% (2 out of 8) blood samples cultured. *K. pneumoniae* also had a prevalence of 23.0% (13 out of 56) sputum samples cultured. The highest number of bacteria was observed in sputum samples, followed by urine and blood, according to sample size (Data not shown in Table).

DISCUSSION

This study investigated the prevalence of different bacteria in blood, sputum, and urine at a tertiary care hospital in Kathmandu, Nepal. Three out of four (i.e. 74.0%) cultured urine samples from UTI patients indicated *E. coli* as the causative organism in our study. Similar prevalence was reported in earlier studies. For example, Ganesh *et al.*[14] reported a 58.7% prevalence of *E. coli* while investigating 1,599 urine samples UTI patients. Similarly, comparable results were reported by Das *et al.*[15] in children aged between 1 and 15 years in Biratnagar city of Eastern Nepal. They found a 59.4% prevalence of *E. coli* in cultured urine samples. In contrast, a recent study showed a 90.4% prevalence of *E. coli* among 135 UTI patients at Dhulikhel Hospital, Nepal.[16] Similarly, Rai *et al.*[17] (2008) reported a 93.3% prevalence of *E. coli* among 1,800 urine samples evaluated at Kanti Children's Hospital in Kathmandu, Nepal. This discrepancy in the prevalence of *E. coli* could be attributed to differences in population characteristics, methodologies and levels of hygienic awareness. As described earlier, MMMCTH is a government-funded, self-governing hospital primarily providing primary healthcare to underprivileged and marginalized populations at an affordable cost. Visitors to this hospital may overrepresent from underprivileged, low socio-economic status, and marginalized populations seeking affordable care. Poor personal hygiene, inadequate household sanitation, and the use of contaminated water for bathing or washing are being frequently reported among low socio-economic clusters,[18] which may partially explain the high prevalence of *E. coli*. However, the lack of information on these behavioural and lifestyle characteristics limits our ability to draw conclusions. Therefore, further studies are needed to confirm these findings.

Similarly, one-fourth of the cultured blood samples indicated the presence of *K. pneumoniae* and *S. aureus* (25.0%; 2 out of 8 blood samples) and *K. pneumoniae* (23.0%; 13 out of 56 sputum samples). Some earlier studies have reported similar frequencies. For instance, 21.6% growth positivity for *K. pneumoniae* was reported among hospitalized patients in Sylhet, Bangladesh.[19] However, a bit low occurrence (i.e. 12.3%) of *K. pneumoniae* in cultured blood samples was also reported for from Duhok City in Kurdistan Region of Iraq.[20] Similarly, 14.6% prevalence was reported in cultured clinical samples from Om Hospital and Research Centre, Kathmandu, Nepal.[21] Such a discrepancy in the prevalence of *Klebsiella* spp. might be due to the variability in sampled population characteristics.[22]

In this study, most of the sputum samples (i.e. 65.0%) were mainly from males compared to females, while the majority of the urine samples (54.0%) were from females compared to males. The occurrence of UTI was higher in women, likely due to anatomical differences, hormonal effects, and behaviours of females.[23] Similarly, a higher number of sputum samples from males compared to females is realistic. The overall prevalence of 'any tobacco use', 'tobacco smoking', and 'tobacco chewing' were consistently reported to be significantly higher among males compared to females.[24] However, further studies are needed to confirm this association.

This study indicated that gentamicin was the most effective antibiotic for bacteria isolated from blood, sputum, and urine. Previous studies have also reported higher susceptibility to gentamicin against different bacteria. For instance, a 91.3% susceptibility to gentamicin was reported in 745 urine samples in a tertiary care children's hospital in Nepal.[25] Similarly, a sensitivity range of 72.6% to 91.2% was reported when examining 7,433 clinical samples from inpatients and outpatients at Tribhuvan University Teaching Hospital, in Kathmandu.[26] Moderate susceptibility (i.e. 79.6%) to gentamicin was also reported when investigating 3,149 samples from northeast Ethiopia[27] and 74.5% susceptibility was reported from tertiary care centre in Nepal.[28] Yet, such susceptibility also ranges from 3.6% to 73.3% as reported by recent systematic review.[29] This discrepancy might be due to variations in study populations, including differences in demographics, nutritional status and genetic backgrounds attributed to ethnicity. However, further studies are needed to confirm these findings.

Although the small sample size limits our ability to generalize, this study indicated a concerning number of antibiotics being resistant. *E. coli* emerged as the main culprit for UTI, particularly among females. Gentamicin demonstrated the most promising efficacy against bacterial infections, offering hope in the fight against various infectious diseases. However, bacteria may develop resistance to gentamicin, as observed with other antibiotics, if its use is not regulated properly. This could pose a serious public health problem in the future. Hence, strict regulations regarding antibiotic prescription - preferably after sensitivity testing and consumption should be enforced. Additionally, comprehensive health education programs should be implemented to minimize the risk of superbugs for future generations.

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