Colistin resistance among Gram-negative isolates in Nepal: A review

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ABSTRACT
Background
Colistin resistance among Gram-negative isolates is a tremendous public health problem, and there are very few studies in Nepal about these pathogens. Hence, this review provides comprehensive data on colistin resistance among Gram-negative isolates from various samples in Nepal.

Methods
Articles reporting colistin resistance among various Gram-negative isolates from Nepal before July 2019 were selected; analyzed and relevant data was collected.

Results
Colistin resistance was low among clinical isolates (less than 6%) in comparison to food and animal isolates (up to 69%). A wide variety of clinical isolates were colistin-resistant in comparison to food and animal isolates. Many of these isolates were highly drug-resistant and also harbored various drug-resistant determinants.

Conclusion
Increased colistin resistance among Gram-negative pathogens is a serious concern. Screening of these isolates in clinical settings, animal farms, and food industries, as well as cautious use of colistin in both clinical and animal farms, is imminent.

Keywords
Clinical isolates, colistin resistance, Gram-negative isolates, multi-drug resistant, Nepal
Background
Colistin, even though it is highly effective against many Gram-negative bacteria, its general clinical use is limited due to its adverse side effects on kidney and nerve cells. However, this antibiotic is increasingly used as a last-line drug for the treatment of multi-drug resistant Gram-negative isolates [1]. Colistin causes bacterial cell lysis due to its binding with anionic molecules, i.e., lipopolysaccharide located on the outer membrane of Gram-negative bacteria [2]. Colistin is one of the antibiotics showing effectiveness for treating multidrug-resistant Enterobacteriaceae, producing carbapenemases like Metallo-β-lactamase (New Delhi Metallo-β-lactamase) and serine carbapenemases (Klebsiella pneumoniae carbapenemase) as well [3]. The rise of resistance against carbapenem in the last decade has resulted in the extensive use of colistin combination therapy as a significant treatment option. However, the rise of resistance against colistin among Gram-negative pathogens may lead to infections that are extremely difficult to treat using antibiotics [4].

In Nepal, the clinical, food, and food animal isolates are increasingly becoming colistin-resistant due to clinicians' imprudent use of the antibiotic and uncontrolled use of colistin in animal farms as food preservatives and as an animal growth promoter. This threat is surging day by day while the investigation of such pathogens is very insufficient. To fill this gap, we conducted this review to know the prevalence and other characteristics of colistin-resistant bacterial isolates in Nepal.

Materials and methods

Literature search
A systematic literature search was carried out for colistin-resistant Gram-negative bacterial isolates in Nepal using various electronic databases (Pubmed and NepJol). In addition to published articles, unpublished materials were also analyzed. The following keywords were used in the literature search for published articles: colistin resistance, Gram-negative isolates, Nepal, multi-drug resistant bacterial isolates, extremely drug resistance, and polymyxin resistance.

Inclusion criteria
Articles which included the following information in abstract and text were selected:
i) reported colistin-resistant Gram-negative isolates from Nepal,
ii) included various features of colistin-resistant isolates like bacterial isolate, prevalence, study period, source specimen, study site, antibiotic susceptibility profile, and presence of other resistance genes.

Exclusion criteria
The following were the exclusion criteria.
i) articles lacking all or most of the variables mentioned above,
ii) abstract only articles,
iii) articles not in English, and
iv) meta-analysis.

Figure 1: Circular representation of the IncX4 plasmid containing the mcr-1 gene from colistin sensitive E. coli.

mcr-1 sequence is represented in green colour. IS10R insertion sequence, which is interrupting the mcr-1 gene is in red colour. Arrows show open reading frames (ORFs). Nucleotide positions are in numbers.

Figure adopted from Terveer et al [1]
Data extraction

The following variables were extracted from the selected studies. Bacterial species that are resistant to colistin, the incidence of resistance, research period, site of study, drug resistance pattern, co-existence of other antibiotic resistance genes, type of sample, and method of detection of colistin resistance.
Results
Colistin resistance among clinical isolates was first reported in 2012 among Providencia rettgeri at Tribhuvan University Teaching Hospital. Then on various Gram-negative isolates have been detected with no sensitivity against it. The prevalence range of colistin resistance among the clinical Gram-negative isolate was 1.3 - 5.24% (Table 1). This resistance mechanism is common among Acinetobacter, E. coli, Klebsiella, and Providencia, respectively. Many of these isolates also co-produced other resistance genes like carbapenemases, beta-lactamas, Aminoglycoside adenyl transferase, aminoglycoside resistance methylase, etc.

Colistin resistance was more prevalent in animal isolates in comparison to clinical isolates. Colistin resistance is common among animal E. coli isolates. The prevalence of colistin resistance was 22.8-69% in E. coli and 10.7 % in Salmonella spp, respectively (Table 2). E. coli isolates obtained from chicken cloacal swabs co-produced many resistance genes. Though there is no information about the susceptibility pattern of all of these isolates, studies that included this information reported high drug resistance of these isolates (Table 2).

Discussion
Colistin resistance in clinical, food, and food animal isolates is a tremendous public health problem worldwide. This mechanism is increasing gradually both in clinical and animal isolates. The major cause of this resistance is the use of colistin in animal farms as an animal food preservatives and growth promoters. Rise of colistin resistance among animal isolates is a severe threat to public health as these isolates can easily infect the peoples working on animal farms and related industries. Such isolates may also spread to water sources as well as act as a reservoir on the environment. Resistance to this last line drug causes enormous treatment difficulties in patients suffering from MDR and XDR isolates as colistin is the drug of choice for their treatment.

The prevalence of colistin resistance among clinical Gram-negative isolates was below 6%. This finding is comparable with the findings of Dalmolin et al. as the average prevalence of colistin resistance was around 7% among E. coli, Klebsiella pneumoniae, and Enterobacter spp [14]. While the prevalence was significantly different in comparison to this study as the prevalence was around 10 % among E. coli, Klebsiella, and Pseudomonas in India [15]. Likewise, the rate of resistance was only 0.67 % among Enterobacteriaceae in Spain [16]. These alterations in prevalence may be due to the difference in the study period, differences in the detection method, the difference in colistin prescription pattern, etc. In Nepal, colistin resistance was higher in Acinetobacter, E. coli, and Klebsiella spp, followed by Providencia, Pseudomonas, Enterobacter, and Citrobacter spp. This result indicates that many significant Gram-negative isolates pose mechanism for colistin resistance. Various other authors have also reported colistin resistance among a variety of Gram-negative isolates including Acinetobacter and Pseudomonas [17]. E. coli, K. oxytoca, K. pneumoniae, Acinetobacter iwoffii, E. agglomerans, Enterobacter cloacae, P. putida, and Pseudomonas aeruginosa [18], Klebsiella, E. coli, Enterobacter, and Acinetobacter [19], and Klebsiella [20].

Many colistin-resistant clinical isolates were resistant to a wide variety of antimicrobial agents, including penicillins, cephalosporins, monobactams, carbapenems, aminoglycosides, quinolones, nitrofurans, etc. The resistance of these isolates against a wide variety of conventional drugs is reported worldwide. Colistin resistant K. pneumoniae from Pakistan was resistant to twenty-three antimicrobial agents from ten antimicrobial groups while it was sensitive only to tigecycline [21]. Similarly, colistin-resistant Acinetobacter isolates were not susceptible to imipenem, meropenem, ampicillin-sulbactam, ciprofloxacin, gentamicin, and amikacin [22]. Very similar antibiotic resistance pattern is reported by other authors as well [23, 24, 25]. Some of these isolates were also positive for many other resistant determinants, including Metallo-beta-lactamase, oxacillinase, beta-lactamase, etc. conferring resistance to a wide array of antibiotics. Production of other resistance genes by these isolates is quite common as there are reports co-production of mcr-1, NDM-1, CTX-M-14, CTX-M-15, CTX-M-55, and TEM-1 by colistin-resistant E. coli isolates from China [25], gyrA, aadA1, TEM, strA, ampC, mcr-1, etc. by highly virulent E. coli isolates from Qatar [24]. Likewise, colistin nonsusceptible Klebsiella pneumoniae isolates were positive for SHV, TEM, CTX-M-1, OXA-1, CMY-16 genes [26].

Incidence of colistin resistance was comparatively higher among animal isolates concerning clinical isolates as up to 69 % E. coli isolates recovered from milk were resistant against colistin. Various studies have reported a high proportion of colistin resistance among various bacterial isolates obtained from animal sources. The study of Liu et al. [27] reported that 76.9 % of pig E. coli isolates were resistant against colistin. In China, the colistin resistance rate was 54.25%, 35.96%, and 26.92% among E. coli isolates from pig, chicken, and cattle fecal swabs, respectively [28]. Excluding the huge colistin resistance in milk E. coli isolates, the prevalence range among E. coli and Salmonella was within 10-27%. Very matching colistin resistance rate is indicated by the study of Huang et al. as the incidence was 24.1% on pig farms, 24.3% at pig slaughterhouses, and 14% and 9.5% at chicken farms and slaughterhouse respectively [29]. E. coli was the major isolate among various colistin-resistant bacterial isolates obtained from various animal samples. The predominance of E. coli isolates resistant to colistin is also reported earlier [30, 31]. In Nepal, Salmonella was also...
Resistant to colistin in addition to E. coli. This resistance mechanism has been reported in Salmonella [32], Klebsiella pneumoniae in Iran [33], Shigella flexneri in China [34].

Meat samples, milk, and cloacal swabs of food animals were the source of these isolates in Nepal. Such isolates have been detected from various birds, pigs, chicken meat, pork, and vegetables [31]. These isolates are limited not only in Gram-negative pathogens, but they have also been reported among Staphylococcus and Bacillus spp from mastitis positive cattle in Nepal [35]. This widespread dissemination of colistin resistance among a wide variety of bacteria and their high incidence is a significant global challenge. Not only this, but there are also reports zoonotic transmission of colistin resistance to humans [36-38]. Hence, a detailed understanding of these pathogens is necessary to prevent their transmission from animals to humans and prevent their uncontrolled spread among clinical, food, and animal isolates in Nepal.

Conclusion
Colistin resistance has been detected among clinical, animal, and food isolates in Nepal for a few years among Gram-negative isolates. High resistance observed mainly in animal, and food isolates. Resistance to almost all of the commonly used antibiotics is frequent among these isolates in addition to the co-production of various drug resistance genes. It is a great public health concern in Nepal and requires more insight to ascertain the sources of contamination. Antimicrobial resistance modules and rational use of antibiotics in hospitals and at the community level should be included as a part of the education program. Curricula and recommendations for treatment need to be evaluated and reviewed periodically. Public-private partnership approaches should be closely monitored for the rational use of antibiotics based on public interest to promote antimicrobial stewardship. It is also necessary to establish a monitoring program to develop awareness among health professionals. We should be very aware of the use of antibiotics, of health and hygiene practices, of good hygiene, and of limiting the use of antibiotics in animals and farms.

Abbreviations
Extensively drug-resistant (XDR), Minimum Inhibitory Concentration (MIC), Multidrug-resistant (MDR), New Delhi Metallo-β-lactamase (NDM)

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c. Manuscript revision: SPD, AP
d. Final approval: SPD, AP

e. Agreement to be accountable for all aspects of the work: SPD, AP

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