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Prevalence and Antimicrobial Resistance of Bacteria Isolated from Stray Dogs and Cats of District Hyderabad

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ABSTRACT

During present study a total of 100 samples (saliva, tail wound, otitis, skin wound, nasal discharge, fecal samples were collected. The samples were identified by morphological, cultural and biochemical tests. The antibiogram of the isolated species were conducted using disk diffusion method. The results demonstrated that prevalence of bacterial pathogens in saliva was 0.06% in stray cats and dogs of Tandojam and Hyderabad respectively. From tail wound prevalence of 02 (0.2%) in cats of Tandojam and Hyderabad, while in dogs 03 (0.3%) in Hyderabad and 02 (0.2%) in Tandojam was observed. From otitis 1 (0.09%) in cats of Tandojam and Hyderabad, while in dogs 02 (0.18%) in Hyderabad and 01 (0.09%) in Tandojam was observed. From skin wound 03 (0.39%) in cats of Hyderabad region and 02 (0.26%) from Tandojam region, while 02 (0.26%) in dogs of Hyderabad region and 03 (0.39%) from Tandojam region was observed. From nasal discharge 01 (0.05%) cats and dogs of Hyderabad and Tandojam regions were observed. From fecal sample 01 (0.07%) in cats and dogs of Hyderabad and Tandojam regions was observed. Antibiotic resistance tests revealed that erythromycin and ofloxacin exhibited high resistance against *Staphylococcus aureus*. Ampicillin and gentamycin were identified as highly resistance against *Bacillus cereus. Pasteurella canis* displayed high resistance to ampicillin, enrofloxacin, and ofloxacin. *Escherichia coli* exhibited high resistance to enrofloxacin and ofloxacin. *Micrococcus luteus* showed high resistance to ampicillin and enrofloxacin. *Pseudomonas aeruginosa* and *Streptococcus pyogens* demonstrated high resistance to enrofloxacin. Based on the findings of the current study, it was concluded that the higher prevalence was observed in saliva, tail, otitis, skin, nasal discharge, and fecal samples. The isolates were *Staphylococcus aureus*, followed by *Micrococcus luteus, Escherichia coli, Bacillus cereus, Pasteurella canis, Pseudomonas aeruginosa,* and *Streptococcus pyogens*, respectively.

Keywords: Antimicrobial, Cats, Dogs, Hyderabad, Prevalence, Resistance, Stray dogs

INTRODUCTION:

Antimicrobial selection due to multiple antimicrobial agents used against pathogenic bacteria, the presence of antimicrobial resistance (AMR) in human and veterinary medicine has been a rising concern for decades (Forbes *et al*., 2024). The bacterial strains that cause antimicrobial resistance (AMR) in stray dogs and cats have not gotten as much attention as antimicrobial resistance in livestock. Bacterial infections resulting from canine bites are not uncommon, as their oral cavities harbor a substantial abundance of microbial taxa. Resistant bacteria (including multidrug-resistant) occur in companion animals and many species among them are shared between animals and humans (Marco-Fuertes *et al*., 2022). However, there is growing concern in both veterinary and public health circles over the emergence of antimicrobial resistance (AMR) in stray cats and dogs, particularly in relation to infections like methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *E. coli* (MDR), and *Klebsiella pneumoniae* (Pomba *et al*., 2017). The spread of owned, unowned, and feral populations of cats (Felis catus) is a result of urbanization and human settlement (Hanmer *et al*., 2017). In addition, it is difficult to treat sick stray dogs and cats, this circumstance increases the risk of drug-resistant diseases spreading between stray animals and humans (Chomel *et al*., 2014). Several studies (Walther *et al*., 2012; Robb *et al*., 2017) reported the spread of antibiotic-resistant bacteria, including opportunistic infections like *Staphylococcus spp*., between dogs and their owners. Additionally, cats are recognized to harbor up to 50 zoonosis worldwide (Woinarski *et al*., 2019) and can transmit a wide range of infections (Loss & Marra, 2017; Legge *et al*., 2020). Furthermore, studies have shown that animals close to humans have greater levels of antimicrobial resistance (AMR) than animals in more isolated environments

(Köck *et al*., 2018). It has also been shown that infections are acquired by stray cats and dogs from their owners (Lloyd, 2007). The most common symptoms of infection in cats with the highly pathogenic γ-retrovirus FeLV are immunosuppression or anemia (Hartmann *et al*., 2012; Westman *et al*., 2019). The use of antibiotics in animals breed for meat production has come under intense criticism in the last 10 years (Van Boeckel *et al*., 2015). Several studies have also shown how the amount and frequency of antibiotic usage in cattle is reducing the medications' efficacy when administered to people for medical purposes (Marquardt & Li., 2018). Whereas, majority of dogs and cats have asymptomatic *Campylobacter species* infections, some of them may exhibit severe or moderate diarrhea. Campylobacteriosis is more common in young, neonatal, and chronically infected animals kept in animal rehabilitation facilities (Acke, 2018). Antimicrobial resistance (AMR) in bacterial pathogens is rising and this tendency is a significant factor (Suriyasathaporn *et al*., 2012). Research has revealed the zoonotic potential and associated health effects of *Campylobacter species* infection in dogs and cats, as well as many hazards for fecal shedding of the illness (Pintar *et al*., 2015). On the other hand, there has been comparatively less focus on the use of antibiotics in stray dogs and cats, especially when it comes to the problem of antibiotic resistance in the bacteria. Antibiotics are typically given to stray dogs and cats as part of the therapy for bacterial infections (Guardabassi *et al*., 2004) or as a safeguard against future bacterial infections in the case of virus epidemics (Lappin *et al*., 2017). Abay *et al*., (2014) conducted a study that examined the effectiveness of several techniques for isolating *campylobacter* transmitted by dogs and cats between 2008 and 2009. However, the study failed to provide the facts on the isolates' sensitivity to antibiotics. Critically essential antibiotics used in human medicine are frequently advised for stray dogs and cats, with a high dependence on broad-spectrum drugs such as cephalosporins, fluoroquinolones, and penicillin (Moulin *et al*., 2008). The World organization for Animal Health (OIE) classifies these antibiotics as critically important veterinary medicines, and the World Health Organization (WHO) agrees that they are also critically important for human medicine. Because broad-spectrum antibiotics are convenient and offer comprehensive coverage against a wide range of bacterial infections, veterinarians often choose them as their first choice for treating bacterial infections (Guardabassi *et al*., 2008; Beco *et al*., 2013). But this approach increases the pressure on selection, which eventually results in emergence of unaffected antibiotic bacteria in commensal bacteria such *Pseudomonas aeruginosa* and *Escherichia coli* (Rzewuska *et al*., 2015, Moyaert *et al*., 2019). Antimicrobial drug resistance in animal and human infections, including those of cats and dogs, has been connected to the usage of these antibiotic classes (Adıgüzel & Horvath 2018; Aslantas *et al*., 2019).

MATERIAL & METHODS

Sample collection: The samples were collected and placed into sterile bottles. Subsequently, they were immediately transported to the Department of Veterinary Microbiology, Sindh Agriculture University in Tandojam. This careful handling ensures the preservation of sample integrity during transit and facilitates their safe and timely arrival at the testing facility for further analysis.

Isolation and Identification of Bacteria: During the present study a total of 100 samples were collected from different parts of the Hyderabad District, 50 from dogs and 50 from cats. Swabs from the samples were inoculated onto agar plates, such as Blood, MacConkey's and Nutrient agar to prepare them for bacterial culture. After that, at 37°C, these agar plates were incubated for 24hours. The morphological characteristics of the bacterial isolates were identified, and Gram's staining was used to verify the morphological variations. For further identification, biochemical tests such as the Voges-Proskauer, Simmons Citrate, Catalase, Oxidase, Coagulase, Indole, Triple Sugar Iron, and Nitrate Reduction assays were carried out.

Antibiotic resistance test: Pure bacterial colonies were selected and collected using a sterilized wire loop. These collected colonies were inoculated into 5ml of normal saline. A sterile cotton swab was immersed in this bacterial suspension, rotating it several times against the inner wall of the tube with firm pressure. Following this, the plates were left to air-dry for a period of 3-5 minutes. Antibiotic discs were positioned on the agar surface and gently pressed to ensure complete contact. The plates were inverted and incubated at 37°C for 24 hours. After this incubation period, the plates were examined and the diameters of the zones of inhibition surrounding the antibiotic discs were carefully measured. The following antimicrobials drugs were included in the testing i.e. amikacin, ampicillin, enrofloxacin, erythromycin, neomycin, ofloxacin, gentamicin, kanamycin, tetracycline, penicillin and streptomycin.

Statistical analysis: Statistical data analysis was performed using Excel (Microsoft office, 365), to calculate means.

RESULTS

Prevalence of bacterial isolation from stray dogs and cats: The present study highlighted the prevalence of bacterial organisms stray dog and cat in Hyderabad and Tandojam region. The different samples e.g. Saliva, tail wound, otitis, skin wound, nasal discharge and fecal sample were evaluated for presence of microorganisms (Table 1). The overall prevalence of 35 (35%) of bacterial pathogens was detected in stray dog and cat samples. *Staphylococcus aureus* (67%) was the most common isolate in stray dogs and cats. The next were *S. pyogens* (38%), *B. cereus* (36%), *E. coli* (40%), *P. canis* (38%), *M. luteus* (25%), and *P. aeruginosa* (50%). A total of 40 (55%), 10 (90%), 8

(62.5%), 6 (66.6%), 15 (86.6%), 16 (62.5%), and 5 (80%) times were *S. aureus* isolated and identified from various types of samples, including saliva (n=40), tail (n=10), otitis (n=8), skin (n=6), nasal discharge (n=15), and faecal sample (n=5). In most infections *P. aeruginosa* was the primary cause of contamination; however, in otitis, *P. canis* and *E. coli* were more common. Similarly, it was observed that *S. pyrogens* were the least common in otitis, whereas *M. luteus* was the least common.

Table 1. Prevalence of bacteria isolated from stray dogs and cats

Type of samples	No of samples	*Staphylococcus aureus*	*Streptococcus pyogens*	*Bacillus cereus*	*E.coli*	*Pasteurella canis*	*Micrococcus luteus*	*Pseudomonas aeruginosa*
Saliva	40	22 (55%)	13 (32.5%)	09 (22.5%)	12 (30%)	08 (20%)	07 (17.5%)	14 (17.5%)
Tail wound	10	09 (90%)	07 (70%)	04 (40%)	06 (60%)	04 (40%)	03 (30%)	06 (60%)
Otitis	08	05 (62.5%)	02 (25%)	03 (37.5%)	05 (62.5%)	05 (62.5%)	04 (50%)	04 (50%)
Skin wound	06	04 (66.6%)	03 (50%)	02 (33.3%)	02 33.3%)	05 (83.3%)	01 (16%)	02 (33.3%)
Nasal discharge	15	13 (86.6%)	05 (33.3%)	08 (53.3%)	07 (46.6%)	7 (46.6%)	04 (26.6%)	11 (73.3%)
Fecal sample	05	04 (80%)	02 (40%)	02 (40%)	02 (40%)	02 (40%)	01 (20%)	03 (60%)
Total	100	67 (67%)	38 (38%)	36 (36%)	40(40%)	38(38%)	25(25%)	50 (50%)

Table 2. Number of samples observed from different sources

Sample Type		Cat		Dog		
		Hyderabad	Tandojam		Hyderabad	Tandojam
	No. of samples	No. of positive samples	No. of Positive samples	No. of samples	No. of Positive samples	No. of Positive samples
Saliva	6	1 (0.06%)	1 (0.06%)	6	1 (0.06%)	1 (0.06%)
Tail wound	10	2 (0.2%)	2 (0.2%)	10	3 (0.3%)	2 (0.2%)
Otitis	9	1 (0.09%)	1 (0.09%)	9	2 (0.18%)	1 (0.09%)
Skin wound	13	3 (0.39%)	2 (0.26%)	13	2 (0.26%)	3 (0.39%)
Nasal discharge	5	1 (0.05%)	1 (0.05%)	5	1 (0.05%)	1 (0.05%)
Fecal sample	7	1 (0.07%)	1 (0.07%)	7	1 (0.07%)	1 (0.07%)
Total	50	9 (4.5%)	7 (3.5%)	50	9 (4.5%)	9 (4.5%)

The data illustrates that bacterial pathogens were most prevalent in skin wounds, with a rate of 0.39% for skin wound 3, followed by tail wounds at 0.2%, and saliva at 0.06%. Additionally, otitis showed a prevalence of 0.09%, nasal discharge at 0.05%, and fecal samples at 0.07%. In summary, these findings emphasize the diverse distribution of bacterial pathogens among stray dogs and cats (Table 2).

Presence of bacterial species: The presence of *Micrococcus luteus, E. coli, Bacillus cereus, Staphylococcus aureus, Pasteurella canis, Pseudomonas aeruginosa,* and *Streptococcus pyogens* were identified (Table 3). The findings of this research indicated that a prevalence of *Micrococcus luteus* 25 (25%), *E. coli* 40(40%), *Bacillus cereus* 36(36%), *Staphylococcus aureus* 67(67%), *Pasteurella canis* 38(38%), *Pseudomonas aeruginosa* 50(50%), *Streptococcus pyogens* 38(38%) were recorded from Hyderabad and Tandojam. In comparison, the occurrence of *Bacillus cereus* was found higher than *Micrococcus luteus, E. coli, Bacillus cereus, Staphylococcus aureus, Pasteurella canis*, *Pseudomonas aeruginosa* and *Streptococcus pyogens*

Table 3. Presence of bacterial species in the Dog and Cat

Bacterial species	Number of bacteria	Percentage
Micrococcus luteus	25	25%
E.coli	40	40%
Bacillus cereus	36	36%
Staphylococcus aureus	67	67%
Pasteurella canis	38	38%
Pseudomonas aeruginosa	50	50%
Streptococcus pyogens	38	38%

Antibiotic resistance: This comprehensive analysis provides valuable information regarding the resistance of bacterial isolations to various antibiotics (Table 4). Antibiotic resistance tests revealed that erythromycin and ofloxacin demonstrated high resistance against *Staphylococcus aureus*. Ampicillin and gentamycin were highly resistance against *Bacillus cereus. Pasteurella canis* shown high resistance to ampicillin, enrofloxacin and ofloxacin. *Escherichia coli* showed high resistance to enrofloxacin and ofloxacin. *Micrococcus luteus* showed high resistance to ampicillin and enrofloxacin. *Pseudomonas aeruginosa* and *Streptococcus pyogens* demonstrated high resistance to enrofloxacin.

Table 4. The antibiotic sensitivity of bacteria isolate from stray dog and cat

Bacterial species	Antibiotic discs	Zone around disc	Identification of sensitivity	Efficiency
Staphylococcus aureus	Ampicillin	09mm	+++	Moderate effective
	Amikacin	10mm	+++	Quite effective
	Erythromycin	15mm	++++	High effective
	Enrofloxacin	09mm	+++	Quite effective
	Gentamycin	08mm	+++	Quite effective
	Kanamycin	04mm	++	Moderate effective
	Neomycin	03mm	+++	Quite effective
	Ofloxacin	0mm	-	Resistant
	Penicillin	10mm	+++	Highly effective
	Sterptomycin	08mm	++	Quite effective
	Tetracycline	10mm	+++	Moderately
Bacillus cereus	Ampicilin	10mm	+++	Quite effective
	Erythromycin	10mm	+++	Quite effective
	Amikacin	09mm	+++	Quite effective
	Enrofloxacin	0mm	-	Resistant
	Gentamycin	15mm	++++	Highly effective
	Neomycin	05mm	++	Moderate effective
	Kanamycin	04mm	+++	Quite effective
	Ofloxacin	04mm	++	Moderate effective
	Pencillin	14mm	++	Highly effective
	Streptomycin	08mm	++	Moderate effective
	Tetracycline	09mm	++++	Quite effective
Pasteurella canis	Ampicilin	09mm	+++	Quite effective
	Amikacin	14mm	++++	Highly effective
	Erythromycin	13mm	++++	Highly effective
	Enrofloxacin	0mm	-	Resistant
	Gentamycin	11mm	++++	Highly effective
	Kanamycin	08mm	+++	Quite effective
	Neomycin	09mm	+++	Quite effective
	Ofloxacin	05mm	++	Moderate effective
	Pencillin	0mm	-	Resistant
	Streptomycin	0mm	-	Resistant
	Tetracycline	10mm	+++	Quite effective
Escherichia coli	Ampicillin	08mm	+++	Quite effective
	Amikacin	09mm	+++	Quite effective
	Erythromycin	07mm	+++	Quite effective
	Enrofloxacin	10mm	+++	Quite effective
	Gentamycin	14mm	++++	Highly effective
	Kanamycin	03mm	++	Moderate effective
	Neomycin	05mm	++	Moderate effective
	Ofloxacin	15mm	++++	Highly effective
	Penicillin	0mm	-	Resistant
	Sterptomycin	06mm	+++	Quite effective
	Tetracycline	07mm	+++	Quite effective
Micrococcus luteus	Amikacin	14mm	++++	Highly effective
	Ampicillin	10mm	+++	Quite effective
	Enrofloxacin	0mm	-	Resistant
	Erythromycin	15mm	++++	Highly effective
	Gentamycin	08mm	+++	Quite effective
	Neomycin	08mm	+++	Quite effective
	Kanamycin	08mm	+++	Quite effective
	Penicillin	0mm	-	Resistant
	Ofloxacin	10mm	+++	Quite effective
	Sterptomycin	08mm	+++	Quite effective
	Tetracycline	10mm	+++	Quite effective
Pseudomonas aeruginosa	Amikacin	07mm	+++	Quite effective
	Ampicillin	09mm	+++	Quite effective
	Enrofloxacin	07mm	+++	Quite effective
	Erythromycin	15mm	++++	Highly effective
	Gentamycin	07mm	+++	Quite effective

	Neomycin	09mm	+++	Quite effective
	Kanamycin	06mm	+++	Quite effective
	Ofloxacin	07mm	+++	Quite effective
	Penicillin	0mm	-	Resistant
	Streptomycin	07mm	+++	Quite effective
	Tetracycline	08mm	++	Moderate effective
Streptococcus pyogens	Amikacin	06mm	++	Moderate effective
	Ampicillin	09mm	+++	Quite effective
	Enrofloxacin	03mm	++	Moderate effective
	Erythromycin	14mm	++++	Highly effective
	Gentamycin	07mm	+++	Quite effective
	Neomycin	0mm	-	Resistant
	Kanamycin	09mm	+++	Quite effective
	Penicillin	10mm	+++	Quite effective
	Ofloxacin	06mm	+++	Quite effective
	Sterptomycin	07mm	+++	Quite effective
	Tetracycline	09mm	+++	Quite effective

DISCUSSION

The stray dog and cats had been well known for their role in transmission of infections around the world. It is important to observe that many diseases possess major health problems in stray dogs and cats worldwide (adaszek *et al*., 2009). Most dominant isolates from saliva, tail, otitis, skin, nasal discharge were *Staphylococcus aureus* followed by *Streptococcus pyogens*, *Bacillus cereus, E.coli, Pasteurella canis, Micrococcus luteus* and *Pseudomonas aeruginosa*, respectively. Similar results were supported by (Griffin & Holt, 2001) reported the most common aerobic isolates were *Enterococcus species (15%)*, *Staphylococcus intermedius (20%),* coagulase-neagtive *Staphylococi* (13%) and *Escherichia coli* (13%). Higher prevalence of Gram-positive bacteria compared to Gram-negative bacteria in the ocular microbiota was observed (Verdenius *et al*., 2024), the most found isolates were Staphylococcus sp., (Farghali *et al*., 2021), followed by Streptococcus spp. and Bacillus spp. (Hamzianpour *et al*., 2022). Respiratory infections are common in dogs. A varying flora of bacterial pathogens is normally present in the respiratory tract of the canines without causing any clinical signs (Kalhoro *et al.,* 2019). In most cases, the bacteria that cause respiratory infections in stray dogs and cats include *Escherichia coli, Streptococcus species.* and *Staphylococcus species* strains (Attili *et al*., 2021). The most common pathogens isolated from pets were *Staphylococcus intermedius* (23%), *E. coli* (18%), non-lactose fermenting coliforms (14%) and *Pseudomonas spp*. (14%) (Kelly & Colon 1992). In a study by Talen *et al*. (1999) there was a predominance of *Staphylococcus aureus* and *Pasteurella canis*. This is consistent with our results which showed that *Pasteurella canis* and *Staphylococcus aureus* were the most prevalent microorganisms. During present study it was observed that *Staphylococcus aureus* (67%) was the most common isolate followed by *Escherichia coli* (40%), *Pseudomonas aeruginosa* (50%), *Streptococcus pyogens* (38%), *Pasteurella canis* (38%), *Micrococcus luteus* (25%) and *Bacillus cereus* (36%) isolated from saliva, tail, otitis, skin, nasal discharge, and feces, 40 (55%), 10 (90%), 8 (62.5%), 6 (66.6%), 15 (86.6%), 16 (62.5%), and 5 (80%) times, respectively. This is comparable to earlier findings published by Rehman *et al*., (2003) that demonstrated the dominating etiological role of *S. aureus* (33%), followed by *E. coli* (18.9%) *Pseudomonas aeruginosa* (9.6%) and *Streptococcus species* (9.2%). Like our findings (Padhy *et al*., 2014) observed that *S. aureus*, *P. aeruginosa*, *E coli, P. canis, B. cereus, M. luteus* were persistently causing infections in stray dogs and cats. Whereas, Thomas, (2008) observed that bacteria, such as *Staphylococcus*, are rarely isolated alone and are usually found in conjunction with other bacteria to cause infections. The synergism of bacteria might lead to greater pathogenesis than individual inputs. This is in accordance with (Padhy *et al*., 2014) who reported that the most common isolation from traumatic wounds was *Staphylococcus* (65.7%). *S. intermedius* is the most prevalent microorganism found in stray cats and dogs. *S. intermedius* may be prevalent because it lives commensally on the skin and mucous membranes of stray cats and dogs (Urumova *et al*., 2012). However, *P. aeruginosa* (75%) was found to be predominant isolate from fecal sample which shows the similarity with previous reports in human beings (Masaadeh & Jaran., 2009). *Staphylococcus* was frequently isolated from all samples. These results align with (Rehman *et al*., (2003), who highlighted the significant role of *S. aureus* (33%) and *E. coli* (18.9%) in similar infections. Padhy *et al*. (2014) demonstrated that *S. aureus, P. aeruginosa, E. coli, P. canis, B. cereus*, and *M. luteus* persistently caused infections in stray dogs and cats. This suggests that infections in stray animals are often polymicrobial, containing pathogens prevalent in chronic infections. (Thomas, 2008) noted that *Staphylococcus* tends to occur synergistically with other bacterial infections. This is supported by (Padhy *et al*., 2014), who reported *Staphylococcus* (65.7%) as the most predominant isolate from traumatic wounds. (Urumova *et al*., 2012) reported *Staphylococcus intermedius* as the most prevalent bacteria in stray dogs and cats, possibly due to its occurrence as a commensal on the skin and mucous membranes of these animals. Furthermore, *P. aeruginosa* (75%) was identified as the predominant isolate in fecal samples that resemble the previous reports in humans (Masaadeh & Jaran, 2009). An explanation for the above results might be that the microbial isolates have strong infections that can be influenced by numerous factors such as localization, depth of affected tissues, tissue perfusion, quality, intensity and strength of host antimicrobial immune response (Thomas, 2008). Our research showed that ofloxacin and erythromycin were highly resistant to *Staphylococcus aureus*. *Bacillus cereus* was highly resistant to ampicillin and gentamycin. Ampicillin, enrofloxacin, and ofloxacin were extremely resistant to *Escherichia coli*. *Micrococcus luruesu* was highly resistant to both ampicillin and ecrofloxacin. It has been shown that *Streptococcus pyogens* and *Pseudomonas aeroginosa* are resistant to enrofloxacin. These results agree with the findings by (Bassessar *et al*., 2013) who reported that the most resistant antibiotic was gentamicin, followed by amoxicillin, ciprofloxacin, and enrofloxacin. *Staphylococcus species* were resistant to gentamicin and enrofloxacin but generally resistant to oxytetracycline and lincomycin (Shambulingappa *et al*., 2010).

CONCLUSION

It is concluded that the major isolates from stray dogs and cats were *E. Coli*, *Proteus* species, *S. aureus*, and *S. pyogens* and majority of the isolates were highly resistant to the gentamicin. The improper use of antimicrobials in animals may result in antimicrobial resistance, thus establishing a potential risk to human health.

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AUTHORS' CONTRIBUTION:

Hsnain Ali, Dildar Hussain Kalhoro and Shahid Hussain Abro designed the experiment. Hasnain Ali and Dildar Hussain Kalhoro conducted the experiment. Hasina Baloch, Muhammad Saleem Kalhoro and Waheed Ali Kalhoro provided critical guidance and oversight throughout the research process. Hassan Ali, Fateh Muhammad Gad, Kalsoom Rind and Sumbal Zain Khosa carried out data analysis. Asad Ullah Marri edited the manuscript and improve English language. All authors have read and approved the final manuscript.

AUTHORS' CONFLICT INTEREST:

The authors have no conflict of interest to declare.

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