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Antibiotic Resistance Profiling of Pseudomonas Species Isolated from Cloacal Swab of Domestic Pigeons

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ABSTRACT: *Antibiotics are used to treat a number of bacterial infections. However, overuse or misuse of antibiotics has raised serious concerns against antibiotic resistance amongst bacteria. Hence, antibiotics are becoming inefficient in treating bacterial infections leading to an increase in mortality rate worldwide. The domestic animals especially birds are a major source of transmission of antibiotic resistant bacteria in human through excrement and cause bacterial diseases in human. The aim of the present study was to assess the efficacy of different antibiotics prior to their prescription as a measure to prevent antibiotic resistance in bacteria. For this 120 cloacal swab samples were collected from the domestic pigeons of District Narowal to isolate Pseudomonas sp. and assess the efficacy of different antibiotics prior to their prescription as a measure to prevent antibiotic resistance in bacteria. Antibacterial activities were evaluated by performing antibiotic susceptibility pattern of Pseudomonas isolates against 18 commercially available antibiotic discs [Trimethoprime (TMP), Clarithromycin (CLR), Gentamicin (GEN), Chloramphenicol (C), Ampicillin (AM), Streptomycin (S), Kanamycin (K), Nitrofurantoin (F), Amoxicillin (AX), Tazobactam (TPZ), Imipenem (IPM), Meropenem (MEM), Levofloxacin (LEV), Nalidixic acid (NA), Ceftriaxone (CRO), Amikacin (AK), Tetracycline (TE) and Ciprofloxacin (CIP)] by using Kirby-Bauer disc diffusion method. Amongst these antibiotics, notably Pseudomonas sp. showed highest sensitivity to Clarithromycin (93.94%), Ampicillin (100%), Amikacin (93%) and Nalidixic (100%). This study established a general antibiotic resistance pattern of commercially used different antibiotics for commonly encountered clinical isolates. Moreover, antibiotics susceptibility tests (AST) should be carried out prior to prescribing antibiotics to the patient. Additionally, the antibacterial activities of local clinical isolates and change in bacteriological profile due to indiscriminate use of antibiotics associated with appearance of multiple drug resistant strains should be evaluated. It was concluded that preventive measure and their implementation is quite necessary to control antibiotic resistance and domestic pigeons can be a carrier of Pseudomonas species and can transmit through their fecal material to humans and other animals.*

Keywords: *Antibiotic resistance, pseudomonas, Antibacterial susceptibility, cloacal swab*

INTRODUCTION

Antibiotics are the foremost antibacterial chemicals for curing bacterial infections and are broadly used in the treatment and elimination of bacterial diseases (Anupurba et al., 2003). They perform their activity by destroying or inhibiting the growth of bacteria. Antibiotics are not only effective in fighting against microbial infections but also effective in controlling the bacterial infections (Baddour et al., 2006). However, overuse and misuse of antibiotics, poor hygiene and contamination control mechanisms had led to antibiotic resistance in bacteria (Shaikh, 2017). Nowadays, infectious pathogens in wild life have become increasingly important throughout the world, as they have substantial impacts on human health and agricultural production (Kabir, 2016). Transmission of various bacterial pathogen from free living birds to humans have been reported such as *E. coli, Pseudomonas aeruginosa*, *Klebsiella* spp., *Salmonella* spp., *Pasteurella* spp., *S. aureus* and *Proteus* spp. were recovered from hoopoe, ibis, sparrow, doves and quails with variable rates (Effat and Moursi, 2005; Hedawy and El-Shorbagy, 2007).

Various species of free living birds, because of their propensity to nest and roost near human activity, may harbor and disseminate various species of bacterial microorganisms to domestic birds and animals (Abdallah and Khalil, 2016). Undomesticated pigeons are classified under the species *Columba livia* and belonging to the Columbidae family, and order Columbiformes. The present population of this species found in most cities are originated from caged and domesticated pigeons at least five thousand years ago in their native territory (Vasconcelos et al., 2018). Public initiated to take care for these birds and gave them food and provided a favorable environment for these birds to adapt. Currently, undomesticated pigeons are found in large numbers at public locations, and often in close contact with man (Gasparini et al., 2017).

Pseudomonas aeruginosa is the most prevalent bacterial species. It can be highly pathogenic causing 50-100% morbidity in experimentally inoculated 4 week old chicklings (Pike et al., 2017). *Pseudomonas aeruginosa* is a Gram negative, medium sized (0.5- 1.0 x 1.5-5.0µm) bacteria that grows on common culture media usually producing a water soluble green pigment chemically made-up of fluorescein and pyocyanin and a specific plummy odor can often be recognized (Degaim et al., 2019). *Pseudomonas aeruginosa* is toxins producing pathogen which causes infections in stages: bacterial adherence, colonization, invasion and dissemination and systemic of toxemic disease (Prince et al., 1991).

Minimum 60 various types of human pathogens have been already isolated from birds. However, research performed with undomesticated pigeons aiming to determine their zoonotic

potential in the prevalence of infections to humans are limited to the isolation of microorganisms, not considering pathological aspects of the infection in these birds (Benskin et al., 2009). In spite of being occurred and frequently isolated, the reports of conveyance of such pathogens to humans from pigeons are very rare, usually occurring with immunosuppressed persons (Haag-Wackernagel et al., 2004).

On the basis of these facts, the aim of the study was to fill knowledge gaps about the prevalence of the pathogen and related virulence factor in pigeon populations. To determine the extent to which pigeons might harbor pathogens and pose a risk to the human population in Pakistan, the pigeons around Narowal were screened for the presence of Pseudomonas as pathogens to public health. Therefore, the study aimed to describe naturally occurring infection of *Pseudomonas aeruginosa* and other species of pseudomonas in domestic pigeons (*Columba livia domestica*) from Narowal, Pakistan and to assess their antibiotic resistance patterns against different antibiotics.

MATERIALS AND METHODS

A cross sectional study was conducted at Microbiology Department Sughra Shafi Medical Complex, Narowal, Punjab Pakistan. A total of 120 samples of domestic pigeons of both gender (Cloacal swabs) were collected from different areas of District Narowal, during a time span of one year. Data was analysed for mean and standard deviation by using SPSS (Statistical Package for Social Sciences) version 24.0.

Sample collection and processing

Each sample was collected in a sterile container. The sample container was labeled with the details of source, date and time of collection and transported to laboratory for analysis within one hour of collection.

Isolation and Identification of *Pseudomonas*

After sample collection, samples were inoculated in Asparagine broth was used as enrichment media for *Pseudomonas* (Silva et al., 2011). Positive result was indicated by green color of broth. Positive samples were again inoculated on asparagine agar plates for the isolation and purification purpose. Firstly incubated plates were incubated for twenty-four hours at 37ºC. After incubation, isolated colonies were further purified using streak plate method.

Antibiotic Susceptibility Testing (AST)

Kirby diffusion disc technique was performed to find out the antibiotic susceptibility pattern of isolated *Pseudomonas*. Following antibiotics were evaluated: Trimethoprime (TMP), Clarithromycin (CLR), Gentamicin (GEN), Chloramphenicol (C), Ampicillin (AM), Streptomycin (S), Nitrofurantoin (F), Amoxicillin (AX), Imipenem (IPM), Meropenem (MEM), Levofloxacin (LEV), Ciprofloxacin (CIP), Ceftriaxone (CRO), Amikacin (AK), Kanamycin (K), Ampicillin (AM), Tetracycline (TE), Nalidixic acid (NA), Tazobactam (TPZ)

Results

Results were interpreted as resistant (R), sensitive (S) and intermediate (I). The antibacterial susceptibility pattern of different antibiotics was summarized in table 1. The antibacterial susceptibility pattern showed that 6.06% (n=2) *Pseudomonas* species were resistant while 93.94% (n=31) were sensitive towards Trimathoprim (TMP) and Clarithromycin (CLR) respectively. The antibacterial activity of antibiotic Gentamicin (CN) against *Pseudomonas* sp. showed antibacterial susceptibility pattern as intermediate 27.27% (n=9), resistance (18.18% n=6) and sensitivity of 54.55% (n=18). It was noticed that antibacterial activity of antibiotic Chloramphenicol (C) against *Pseudomonas* was 18.18% (n=6), 60.61% (n=20) showed intermediate growth and 21.21% (n=7) showed sensitivity. The antibacterial susceptibility pattern of Ampicilin (AM) against *Pseudomonas* revealed 100% (n=33) resistant. The antibacterial susceptibility pattern of Streptomycin (S) had shown that 36.36% (n=12) intermediate growth, 57.58% (n=19) resistance and 6.06% (n=2) shown sensitivity against *Pseudomonas*. The antibacterial activity of antibiotic Nitrofurantoin (F) against *Pseudomonas* was 81.82% (n=27) with resistance and 18.18% (n=6) showed sensitivity whereas no intermediate growth was recorded.

The antibacterial activity of antibiotic Amoxicilin (AX) against *Pseudomonas* showed 100% (n=33) resistance and no intermediate and sensitivity growth was recorded. The antibacterial activity of antibiotic Tazobactam (TPZ) against 36.36% (n=12) were with intermediate growth, 57.58% (n=19) were showed resistance and 6.06% (n=2) showed sensitivity. The antibacterial susceptibility pattern of Imipenem (IPM) had shown that 18.18% (n=6) *Pseudomonas* species showed intermediate growth, 33.33% (n=11) showed resistance and 48.48% (n=16) showed sensitivity. The antibacterial activity of antibiotic Meropenem (MEM) of *Pseudomonas* showed 54.55% (n=18) intermediate growth, 33.33% (n=11) showed resistance and 12.12% (n=4) showed sensitivity. The antibacterial activity of antibiotic Levofloxacin (LEV) against *Pseudomonas* 42.42% (n=14) showed intermediate growth 33.33% (n=11) showed resistance and 24.24% (n=8) showed sensitivity. The antibacterial activity of antibiotic Ciprofloxacin (CIP) against *Pseudomonas* sp. 8.06% (n=2) were showing intermediate 51.52% (n=17) were showing resistance and 42.42% (n=14) were showing sensitivity. The antibacterial activity of antibiotic Ceftriaxone (CRO) against *Pseudomonas* is of *Pseudomonas* 6.06% (n=2) were showing intermediate 93.94% (n=31) were showing resistance and whereas *Pseudomonas* does not shown any sensitivity. The antibacterial activity of antibiotic Amikacin (AK) against *Pseudomonas* sp. was 100.00% (n=33) were showing sensitivity and whereas

Pseudomonas sp. did not show any Resistant and intermediate growth. The antibacterial activity of antibiotic Kanamycin (K) against *Pseudomonas* was 9.09% (n=3) were showing intermediate 36.36% (n=12) were showing resistance and 54.55% (n=18) were showing sensitivity. The antibacterial activity of antibiotic Tetracyclin (TE) against *Pseudomonas* was 6.06% (n=2) were showing intermediate 51.52% (n=17) were showing resistance and 42.42% (n=14) were showing sensitivity. The antibacterial activity of antibiotic Nalidixic Acid (NA) against *Pseudomonas* 93.94% (n=31) were showing resistance and 5.05% (n=2) were showing sensitivity. Whereas, *Pseudomonas* sp. did not show any intermediate growth.

Fig. 1 showed the sensitivity of *pseudomonas* sp. against different tested antibiotics. Amikacin (AK), Trimethoprime (TMP) and Clarithromycin (CLR) were noticed as most effective against concerned bacteria whereas Fig. 2 showed the resistance pattern of *pseudomonas* species against tested antibiotics. Results revealed that majority of tested antibiotic have very low antibiotic efficacy against isolates.

Table 1. Antibiotic resistance profiling of *Pseudomonas* species isolated from cloacal swab of domestic pigeons

Antibiotic	Sensitive	Intermediate	Resistant
Trimethoprime (TMP)	93.94%	ND	6.06%
Clarithromycin (CLR)	93.94%	ND	6.06%
Gentamicin (GEN)	54.55%	27.27%	18.18%
Chloramphenicol (C)	21.21%	18.18%	60.61%
Ampicillin (AM)	ND	ND	100%
Streptomycin (S)	6.06%	36.36%	57.58%
Nitrofurantion (F)	18.18%	ND	81.82%
Amoxicillin (AX)	ND	ND	100%
Tazobactam (TPZ)	6.06%	36.36%	57.58%
Imipenem (IPM)	48.48%	18.18%	33.33%
Meropenem (MEM)	12.12%	54.55%	33.33%
Levofloxacin (LEV)	24.24%	42.42%	33.33%
Ciprofloxacin (CIP)	42.42%	8.06%	51.58%
Ceftriaxone (CRO)	ND	6.06%	93.94%
Amikacin (AK)	100%	ND	ND
Kanamycin (K)	54.55%	9.09%	36.36%
Tetracyclin (TE)	42.42%	6.06%	51.52%
Nalidix Acid (NA)	5.05%	ND	93.94%

Abbreviation: Not Detected (ND)

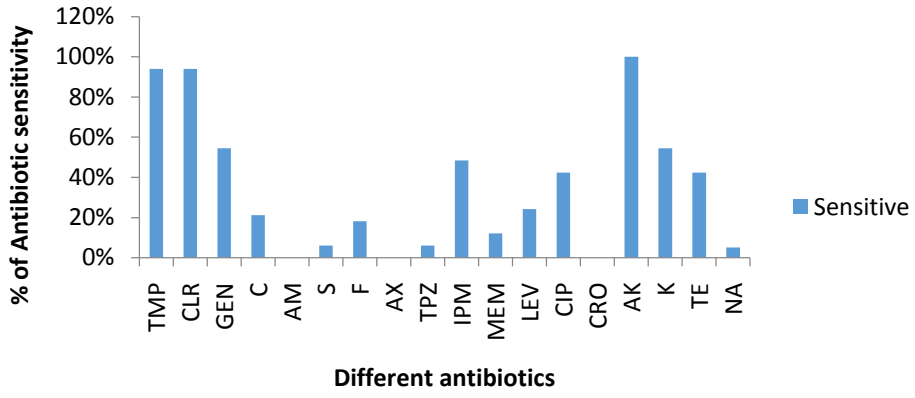

Fig. 1. Comparative sensitivity of different antibiotics against *Pseudomonas*

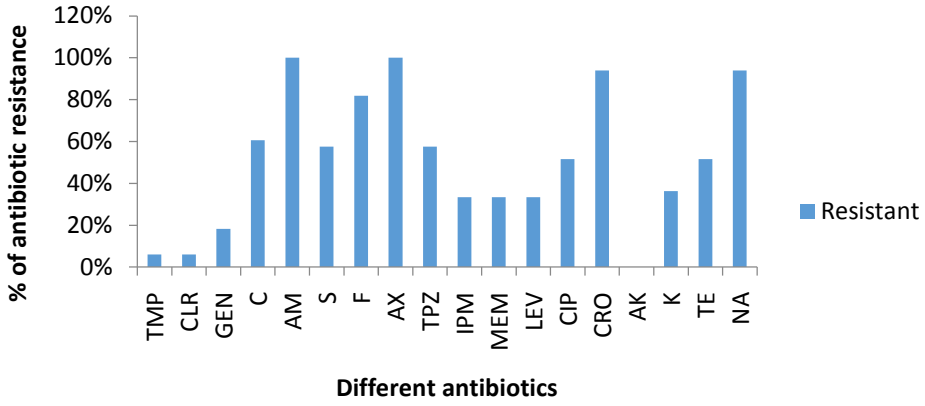

Fig. 2. Comparative Resistance of Pseudomonas against antibiotics

DISCUSSION

In this study, 120 cloacal swab samples were collected from the domestic pigeons of District Narowal. Clinical isolates of *Pseudomonas* sp. were obtained from 33 (27.5%) samples which were identified through conventional culture and biochemical tests. *Pseudomonas* species were the most common bacteria and is found in 7.8% of related species of birds. Aerobic bacteria were reported in the microbiota of the large intestine of many animals as well as humans (Lister et al., 2009). *Pseudomonas* species were also found in birds and under favorable conditions they developed a symbiotic relationship. It may be associated with many infectious conditions e.g. rhinitis, sinusitis, laryngitis, septicemia and hemorrhagic enteritis (Bailey et al., 2009).

This study aimed to test various antibiotics for antibacterial activity against isolated *Pseudomonas species*. Primarily, the purpose was to compare the antibacterial activities of these antibiotics against *Pseudomonas* isolates.

To the best of our knowledge, studies on AMR profiles of these microorganisms in related birds in companionship are not available. There is possibility to transmit infections and resistant traits to other species and also to humans, making it a concern for public health that needs attention. In a previous study, *Pseudomonas aeruginosa* was found in 7% of the birds of prey with blood infections (Vidal et al., 2017) and oral lesions while in our study, the frequency was 25%, even in healthy birds. Mostly, the bacterial strains showed resistance to all the antibiotics while only one showed resistance to gentamicin. In contrast, the above study reported 100% resistance to clindamycin and 21% to gentamicin.

Before selecting an antibiotic for any bacterial infection, the information for antibiotic susceptibility test (AST) should be collected (Arayne et al., 2002). Accurate performance of susceptibility tests were very important to obtain good results in accordance with the condition and treatment (Ryan, 1970). These symbiotic and opportunistic bacteria were critical and its important to have identification of new antibiotics to cope with MDR properties (Pragasam, 2018). AST should be applied before prescribing the antibiotics to patients. Furthermore implementation of preventive measures was recommended for the control and prevention of antibacterial resistance. This study helped in establishing a general antibiotic resistance pattern of currently in use different antibiotics for commonly encountered clinical infections caused by *Pseudomonas sp.* A study is recommended in future to evaluate the antibacterial activities of local clinical isolates and also subsequently identify change in bacteriological profile due to indiscriminate usage of antibiotics associated with the appearance of multiple drug resistant strains.

CONCLUSION

On the basis of this study, it was concluded that domestic pigeons can be a carrier of *Pseudomonas* species and can transmit through their fecal material to humans and other animals. Preventive measures must be taken to avoid transmission. Moreover, Trimethoprime and Clarithromycin were best to use as both showed least resistances as compared to all other antibiotics.

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