

Original Research Article

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Antimicrobial Resistance Profile of Livestock and Poultry of North-Western Punjab

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ABSTRACT

Antimicrobial resistance has made its niche in diverse animal pathogens including protozoa especially in emerging and re-emerging zoonotic pathogens, thus act as one of the most formidable enemy of the society. The study was aimed to document the prevailing antibacterial resistance profile of bacterial isolates from clinical samples (uterine discharge, mastitic milk, direct swabs from throat, nasal and ocular infection) of different animal species (bovine, caprine, canine and poultry). The isolated bacteria are *Pasteurella*, *E. coli*, *Klebsiella*, *Salmonella*, *Pseudomonas*, *Staphylococcus*, *Streptococcus*, *Corynebacterium* and *Bacillus*. Resistance to at least one antimicrobial agent was demonstrated in all the isolates and the highest levels of multidrug-resistant strains were obtained from bovine milk followed by cloacal swabs of poultry birds. *E.coli* was found to be most sensitive to amikacin, ceftazidime and cefotetan; *Staphylococcus aureus* to piperacillin/tazobactam; *Streptococcus* sp to piperacillin/tazobactam, ceftazidime and ticarcillin/clavulanic acid; *Pseudomonas* and *Klebsiella* sp to amikacin; and *Pasteurella multocida* was found to be sensitive to cefotaxime, clindamycin, chloramphenicol and gentamicin. This profile will help in selection of antimicrobials for treatment of animals in the North-Western region of Punjab.

Keywords

Antibacterial
resistance profile,
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Introduction

Antibacterial resistance has become one of the most critical public health problems (Davis and Davis, 2010; Cabello *et al.*, 2016; Robinson *et al.*, 2016a). Injudicious use of antimicrobials in the livestock industry, agriculture and biomedicine has resulted in the

creation of strong selective resistance to antibiotics (Manyi-Loh *et al.*, 2018). This emergence of resistant bacterial strain is a major problem in animal and human medicine (Wegener *et al.*, 1999; Aslantas and Demir, 2016). Profiling of resistant strains prevailing in Punjab in the livestock sector as well as poultry farms will provide for and mitigate the

present problem of injudicious use of antibiotics and public health concern. World Health Organization (WHO, 2016) has emphasized the importance of reducing the incidence of bacterial resistance and production losses to the livestock industry in general and public health significance in particular. To achieve this goal, comprehensive documentation of antimicrobial usage and emergence of resistance among humans and animals is necessary.

Antimicrobials are crucial for maintenance of animal health, animal welfare and food-safety (FAO, 2016; Magouras *et al.*, 2017). Recent reports indicated a steady rise in antibiotic resistance globally among the animals as well as humans (Aslam *et al.*, 2018). Resistant bacterial strains of *Staphylococcus aureus*, *E.coli*, *Salmonella*, *Klebsiella pneumoniae*, *Campylobacter* isolated from cattle, pigs, poultry farms, human hospitals, slaughterhouses, animal handlers, etc are continuously emerging even against higher generation of antibacterials like Quinolones (Zurfluh *et al.*, 2015), Methicillin (Yang *et al.*, 2016), Cephalosporins and many other classes (Gharied *et al.*, 2015; Kashoma *et al.*, 2015; Agabou *et al.*, 2016; Eguale *et al.*, 2016; Lalak *et al.*, 2016; Ibrahim *et al.*, 2016; Papadopoulos *et al.*, 2016; Ranjbar *et al.*, 2016). Under such situations, control of antibiotics use in agricultural farms and livestock industries (Aarestrup, 2015; Vishnuraj *et al.*, 2016; Robinson *et al.*, 2016b), clinics and hospitals (Weinstein, 2001) will play an important role for effective extenuation of the spread of antibiotic resistance.

Antibiogram profile of various bacterial strains present in different ecosystem varies with regards to its geographical location (Sahoo *et al.*, 2012). Detection and documentation of prevalent microbial system

with antibiogram profile is expected to contribute as a biological guide to minimize and limit the injudicious use of antimicrobials and non-essential prophylactic substances in the system (WHO, 2013). Our work has been designed to document the antibiotic resistance profile of prevalent bacterial pathogens causing common infections in livestock, companion animals and poultry of North-Western Punjab regions.

Materials and Methods

Sample collection and preparation

A total of 235 clinical samples from swabs and lesions at different sites (skin scrapes, ocular, nasal and uterine discharges, rectal pinch) mastitic milk and diarrheic samples from cattle, buffalo, sheep, goat, dog and poultry routinely submitted from Veterinary Clinical Complex (VCC) as well as specimens collected separately from the field during the past four years (i.e., years 2014 to 2018) were processed as per standard microbiological protocols.

Microbial isolation and biochemical identification of bacteria

Samples were individually processed for isolation of bacterial pathogens as per routine bacterial isolation method (Markey *et al.*, 2013). Samples were inoculated into brain heart infusion (BHI) broth and incubated at 37°C for 16 hrs. Isolation of single colonies was achieved as per routine streaking method into 10% sheep blood agar and incubated both aerobically and anaerobically in a GasPak system at 37°C for 48 hrs. Single isolated colonies were stained with routine staining procedure to identify Gram stained characteristic and morphology.

Bacterial colonies were subjected to standard biochemical testing for species identification.

Antibiogram

Antimicrobial susceptibility tests were performed using disk diffusion method on Mueller Hinton Agar (MHA) plate following Clinical and Laboratory Standards Institute (CLSI) documents M100-S22 (CLSI, 2019). The following antibiotic discs (HiMedia) were tested-Amoxicillin/Clavulanic acid (100/10mcg, AMC), Ampicillin (AMP), Azithromycin (AZT), Methicillin (MET), Clindamycin (CD), Gentamicin (GEN), Enrofloxacin (%mcg, E), Ciprofloxacin, Nalidixic acid, Nitrofurantoin, Ertapenem, Cefotetan, Cefotaxime, Ceftazidime, Ticarcillin/Clavulanic acid, Piperacillin/Tazobactam, Colistin. Isolates resistant to three or more antimicrobial classes are classified as Multi-Drug Resistant strains (MDRs) according to Magiorakos and coworkers (2012).

Results and Discussion

Microbial isolation and biochemical identification of bacteria

Major pathogenic organisms isolated from the clinical samples ranges from gram negative bacteria such as *E.coli*, *Salmonella*, *Klebsiella*, *Citrobacter*, *Pseudomonas*, *Pasteurella* and gram positive bacteria such as *Staphylococcus*, *Streptococcus*, *Corynebacterium* and *Bacillus* as shown in the table 1.

In mastitic milk samples of dairy animals, *Staphylococcus aureus* (41.25%) was found to be most prevalent followed by *E. coli* (30.00%), *Streptococcus* sp and *Klebsiella* sp (11.25%).

Staphylococcus, *Pseudomonas*, *Corynebacterium* and *Bacillus* species are isolated from skin scrapes, throat swabs and ocular swab samples from cows, buffaloes, horses and dogs. *Staphylococcus aureus* (43.3%) was found to be the most prevalent

isolates from skin scrapings, followed closely by *Pseudomonas* and *Corynebacterium* sp in canines. *Staphylococcus pseudintermedius* was confirmed as the major isolate from canine skin and ear infection, whereas, *Staphylococcus aureus* made up the majority of isolates from mastitic milk samples. However throat and ocular swabs showed *Corynebacterium* (50.0%) and *Staphylococcus* sp (30.0%) as the major isolates in canine samples, respectively.

In uterine discharge samples of caprine, the most prevalent isolates were *E. coli* (28.9%) and *Staphylococcus* species (31.1%). *Pasteurella multocida* (40.0%) was isolated from bovine throat swab samples. *Salmonella* sp. (40.0%), *E.coli* (40.0%) and *Klebsiella* sp. (10.0%) were isolated from cloacal swab samples from a poultry farm with diarrheal outbreak and sudden death.

Antibiogram

The bacterial isolates from various clinical samples showed resistance to antibiotics as shown in the tables (Table 2 and 3; Fig. 1 and 2). Multi-drug resistance was observed with *E.coli* isolates. Gram positive *Staphylococcus* isolates from canine skin infections showed resistance to a majority of the commonly used antibiotics. Other bacterial isolates such as *Streptococcus*, *Corynebacterium*, *Pasteurella*, *Klebsiella*, and *Salmonella* showed resistance percentages ranging from 4% to 50% against various antibiotics commonly used for treatment of infection in animals.

Resistance to at least one antimicrobial agent was demonstrated in all the isolates and the highest levels of multidrug-resistant strains were obtained from bovine milk followed by poultry rectal swabs. Antibiotic resistance was observed to be very high in isolates of *E.coli*, *Pseudomonas* and *Staphylococcus* sp. *E. coli* was found to be most sensitive to amikacin,

ceftazidime and cefotetan; *Staphylococcus aureus* to piperacillin/ tazobactam; *Streptococcus* sp to piperacillin/ tazobactam, ceftazidime and ticarcillin/ clavulanic acid; *Pseudomonas* and *Klebsiella* sp to amikacin; and *Pasteurella multocida* was found to be sensitive to cefotaxime, clindamycin, chloramphenicol and gentamicin.

Many of these isolated organisms are commensals of the skin and the digestive tracts. Gram-negative such as *E.coli* and *Pseudomonas aeruginosa* formed one of the major isolates from mastitic milk samples. Isolation of coliform bacteria such as *E.coli* and *Klebsiella* in milk indicated environmental contamination and unhygienic practice in the farm.

Gram positive bacteria, viz., *Staphylococcus* and *Streptococcus* are one of the most common pathogens responsible for mastitis in milch animals and are responsible for huge economic losses of the dairy industry. Isolation of these gram-positive bacteria in milk indicated unhygienic milking practice on the farm. Many isolates are resistant to antibiotics and can be responsible for a serious problem regarding hygienic milk production, animal and public health.

Staphylococcus sp are commensals of the skin and are the major pathogens for bacterial pyoderma, folliculitis and contagious epidermal collarette in dogs.

Pseudomonas sp are opportunistic bacteria and are found in compromised skin lesions such as burns, animals receiving steroid therapy (immunosuppressed/ immunocompromised), chronic ear infection and pododermatitis in conjunction with certain yeasts such as *Malassezia* sp. In poultry birds, *Salmonella* sp. is one of the most important pathogens causing acute infections.

The result of the study has shown widespread distribution of antibiotic resistant bacterial strains not only in the livestock, but also that of pet animals in Punjab.

Increase in the distribution of antibiotic resistant bacterial strains in various farm animals and as well as pet animals poses greater challenge for public health with regards to transmission of antibiotic resistant pathogens from farm to farm, animals to humans and vice versa (Kruse, 1999; Guardabassi *et al.*, 2004; Phillips *et al.*, 2004). Due to limitations of antimicrobials used for treatment of pets, commercially available antimicrobials are usually used in veterinary practice, such as antibiotics banned in food animals (eg. chloramphenicol), topical ointment for humans *e.g.* mupirocin and fusidic acid) and last-line of antimicrobials in human medicine *e.g.* new generation cephalosporins and fluoroquinolones) (Guardabassi *et al.*, 2004). To ameliorate further increase in emergence of new resistant strains, public awareness, farmer - veterinarian interactions and alternative methods of medications and disease control measures like vaccinations need to be encouraged.

Current knowledge on prevalence of antimicrobial resistance, injudicious use of a particular class or type of antibiotics in farm animals, poultry as well as dogs and cats with focus on the potential implications for human health are very much inadequate in Amritsar region.

This compiled data of resistance chart as per samples received from clinics and field can assist in choosing and avoiding the use of a particular antibiotic in a particular region or farm.

Table.1 Lists of pathogenic microorganisms isolated from clinical samples of buffaloes, cows, sheep, goat, and dogs obtained from TVCC, KCVAS and from field cases

Clinical sample (Total)	Species	Micro-organism(s)	No of positive samples	Percentage positivity (%)
Mastitic milk (80)	Bovine	<i>Escherichia coli</i>	24	30
		<i>Klebsiella sp</i>	10	12.5
		<i>Citrobacter sp</i>	4	5
		<i>Bacillus sp</i>	9	11.25
		<i>Staphylococcus sp</i>	33	41.25
		<i>Streptococcus sp</i>	12	15
		<i>Corynebacterium sp</i>	8	10
Skin scrapings (30)	Canine	<i>Staphylococcus sp</i>	13	43.3
		<i>Pseudomonas sp</i>	9	30
		<i>Corynebacterium sp</i>	8	26.6
		<i>Bacillus</i>	4	13.3
Throat swab (20)	Canine	<i>Corynebacterium sp</i>	10	50
		<i>Pasteurella sp</i>	7	35
		<i>Streptococcus</i>	7	35
Throat swab (10)	Bovine	<i>Pasteurella sp</i>	4	40
		<i>Streptococcus</i>	4	40
		<i>Staphylococcus sp</i>	3	30
Ocular swab (20)	Canine	<i>Staphylococcus</i>	6	30
		<i>Streptococcus</i>	4	20
		<i>Bacillus</i>	5	25
		<i>Pseudomonas</i>	4	20
Uterine discharges/deep vaginal swab (45)	Caprine	<i>Staphylococcus</i>	14	31.1
		<i>Streptococcus</i>	4	8.9
		<i>E. coli</i>	13	28.9
		<i>Pseudomonas</i>	3	6.6
Cloacal swab (30)	Poultry	<i>Salmonella</i>	12	40
		<i>E. coli</i>	12	40
		<i>Klebsiella</i>	3	10

Table.2 The number of antibiotic resistant gram negative bacteria observed per isolates (as per CLSI standard)

Bacteria (=n)	<i>E.coli</i> (49)	<i>Klebsiella</i> (13)	<i>Citrobacter</i> (4)	<i>Salmonella</i> (12)	<i>Pseudomonas</i> (18)	<i>Pasteurella</i> (7)	<i>Corynebacterium</i> (26)
MT 5 mcg	8/49	3/12	-	-	-	1/7	-
AMP 10mcg	34/45	4/13	0/4	1/2	4/15	2/7	8/24
C 30mcg	6/48	2/13	-	-	-	1/7	-
AZM 15mcg	7/49	2/12	-	-	-	3/7	-
TE 30mcg	28/48	6/13	1/4	1/2	9/14	5/7	5/24
AK 30mcg	2/49	1/11	-	-	-	3/6	-
CD 2mcg	7/48	-	-	-	-	0/7	-
GEN 10mcg	6/49	-	-	-	-	2/7	-
EX 10mcg	12/38	3/11	0/4	0/2	4/16	2/7	1/23
CIP 5mcg	17/44	3/13	1/4	1/2	5/18	1/6	2/25
NA 30mcg	3/44	-	0/4	-	-	1/5	-
NIT 100mcg	4/40	-	0/4	-	-	-	-
ETP 10mcg	2/39	-	-	-	-	-	-
CTN30mcg	5/45	-	-	-	-	-	-
CTX 30mcg	4/42	-	1/4	-	-	0/6	-
CAZ 30mcg	6/47	-	-	-	-	0/6	-
TCC 75/10 mcg	2/32	-	0/4	-	-	0/6	-
PIT 30/6 mcg	3/28	-	0/4	-	-	-	-
CL 10mcg	4/41	-	-	-	-	-	-

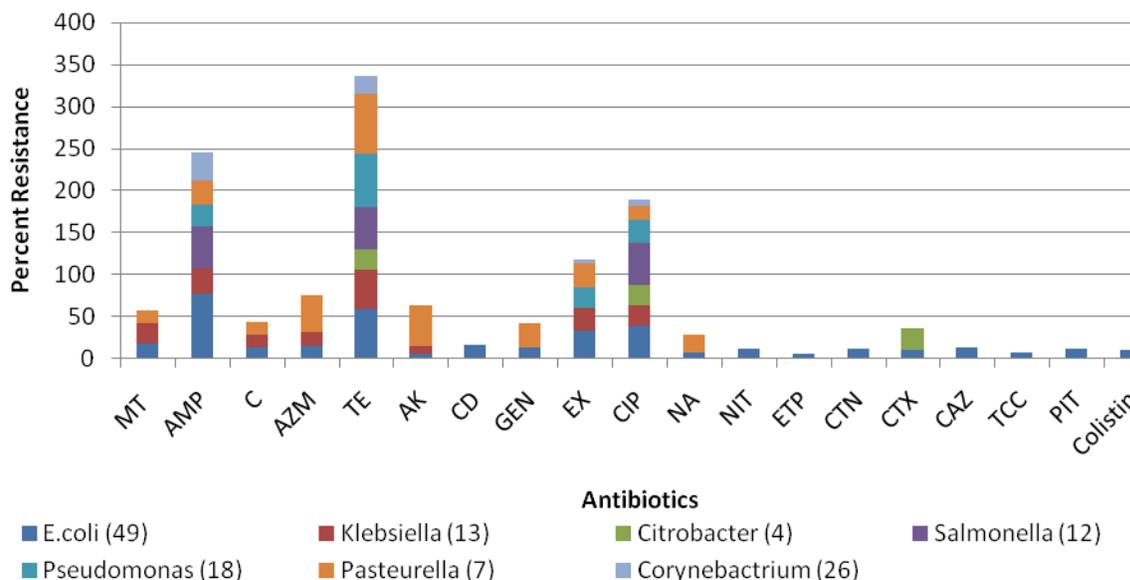
AK = Amikacin, AMP= Ampicillin, AZM = Azithromycin, CTX = Cefotaxime, CTN = Cefotetan, CAZ = Ceftazidime, C = Chloramphenicol, CIP = Ciprofloxacin, CD = Clindamycin, CL = Colistin, EX = Enrofloxacin, ETP = Ertapenem, GEN = Gentamicin, MT = Metronidazole, NA = Nalidixic acid, NIT = Nitrofurantoin, TCC = Ticarcillin/Clavulanic acid, PIT = Piperacillin/Tazobactam.

Table.3 Percentage of antibiotic-resistance pattern of Gram positive bacterial isolates (as per CLSI standard)

Bacteria	AM C	AM P	AZ M	ME T	CD	C	GE N	CI P	CT N	CTX	CA Z	TC C	PIT
<i>Staphylococcus</i> (66)	15	33	24	42	21	23	23	18	23	18	16	12	11
<i>Streptococcus</i> (27)	8	23	15	22	8	12	7	8	14	12	11	6	4

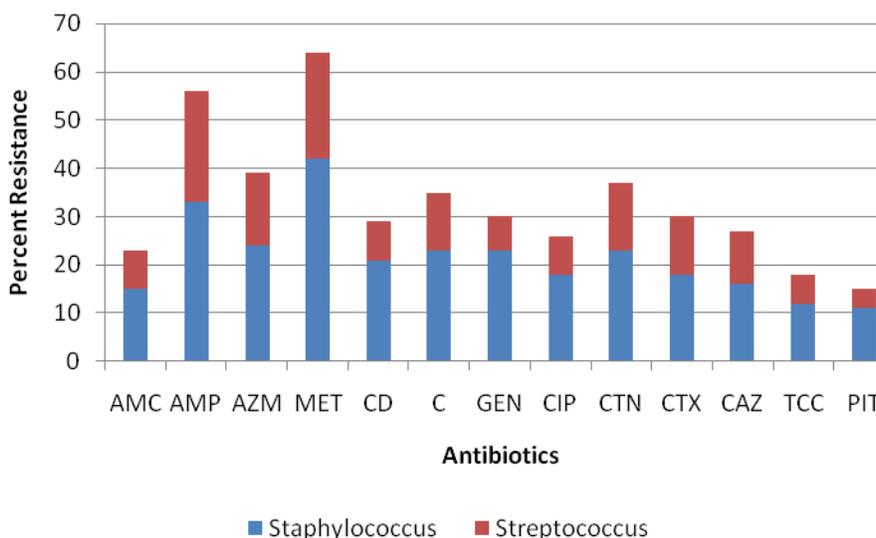
AMC = Amoxycillin/Clavulanic acid, AMP= Ampicillin, AZM = Azithromycin, MET = Methicillin, CD = Clindamycin, C = Chloramphenicol, GEN = Gentamicin, CIP = Ciprofloxacin, CTN = Cefotetan, CTX = Cefotaxime, CAZ = Ceftazidime, TCC = Ticarcillin/Clavulanic acid, PIT = Piperacillin/Tazobactam, CL = Colistin

Fig.1 Percent antibiotic-resistance of gram negative bacterial isolates against commonly used antibiotics



AK = Amikacin, AMP= Ampicillin, AZM = Azithromycin, CTX = Cefotaxime, CTN = Cefotetan, CAZ = Ceftazidime, C = Chloramphenicol, CIP = Ciprofloxacin, CD = Clindamycin, CL = Colistin, EX = Enrofloxacin, ETP = Ertapenem, GEN = Gentamicin, MT = Metronidazole, NA = Nalidixic acid, NIT = Nitrofurantoin, TCC = Ticarcillin/Clavulanic acid, PIT = Piperacillin/Tazobactam.

Fig.2 Percent antibiotic-resistance of *Staphylococcus* sp. and *Streptococcus* sp. against commonly used antibiotics



AMC = Amoxycillin/Clavulanic acid, AMP= Ampicillin, AZM = Azithromycin, MET = Methicillin, CD = Clindamycin, C = Chloramphenicol, GEN = Gentamicin, CIP = Ciprofloxacin, CTN = Cefotetan, CTX = Cefotaxime, CAZ = Ceftazidime, TCC = Ticarcillin/Clavulanic acid, PIT = Piperacillin/Tazobactam, CL = Colistin.

Adoption of appropriate and timely measures like proper drug sensitivity tests, rotational and differential usage of antibiotics and avoidance of continuous and excessive use of specific antibacterial agents for a particular pathogen. Such measures will have long lasting beneficial and economic effects for animal health in general and food safety and public health significance in particular.

Conflict of Interest

The authors declare that there is no conflict of interest to disclose regarding the research work presented here.

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