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Comparative Antimicrobial Activity of Amikacin and Gentamicin on Clinically Important Bacteria

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Abstract

Aminoglycosides discovered 80 years ago are still the drug of choice for combating a range of infections, including those caused by multiple drug-resistant (MDR) strains of bacteria. These bactericidal antibiotics are nephrotoxic and ototoxic and often not permitted for therapeutic use in animals and birds. This study analyzed the comparative *in vitro* antimicrobial activity of amikacin and gentamicin on 517 isolates of potentially pathogenic bacteria isolated from environment and food samples (53), reference strains (11) and from clinical samples (453) of defined ailments in animals (299), humans (85), and birds (69). Gentamicin inhibited 77.37%, and amikacin inhibited 73.11% of the isolates. Of 378 strains of bacteria susceptible to amikacin 13.76% were resistant to gentamicin, and of the 400 strains susceptible to gentamicin 18.50% were resistant to amikacin. A significant ($p < 0.001$) correlation between carbapenem resistance and amikacin (r, 0.264) and gentamicin (r, 0.31) resistance was apparent. Susceptibility to gentamicin and amikacin varied among different bacteria like *Alcaligenes* spp. (15.38%, and 23.08%), *Enterococcus* spp. (37.50%, and 37.50%), *Escherichia* spp. (27.13%, and 24.03%), *Klebsiella* spp. (1.58%, and 21.05%), *Pantoea agglomerans* (24.14%, and 31.03%), *Proteus* spp. (28.57%, and 78.57%), *Pseudomonas* spp. (15.38%, 26.92%), *Salmonella enterica* ssp. *enterica* serovars (16.67%, and 16.67%), *Staphylococcus* spp. (18.81%, and 24.75%), and *Streptococcus* spp. (34.69%, and 44.90%), respectively. Source of the bacterial strains also affected the susceptibility viz., 13.33% and 46.67% of *E. coli* isolates from poultry birds were resistant to gentamicin and amikacin, respectively but none of the *E. coli* isolated from infections in pigs was resistant to gentamicin or amikacin. Bacteria associated with eye, gastrointestinal tract and genital tract infections were more often resistant to amikacin than those associated with otitis and septicaemia. *Proteus, Staphylococcus* and *Streptococcus* species strains showed more resistance to amikacin than to gentamicin but the difference was statistically insignificant ($p, >0.05$). A wide variation in susceptibility of bacterial strains of different species causing various types of infections in animals and birds under different husbandry practices suggests that antimicrobial susceptibility should be conducted before the use of amikacin or gentamicin in therapeutics.

INTRODUCTION

Streptomycin was the first aminoglycoside isolated in 1943 from *Streptomyces griseus* followed by neomycin (from *Streptomyces fradiae*). Though neomycin had better antimicrobial action against aerobic G-ve bacteria than streptomycin, due to its high toxicity its systemic use was formidable. Gentamicin, isolated in 1966 from *Micromonospora purpura*, brought a breakthrough in the treatment of G-ve bacterial infections and then came semisynthetic aminoglycosides amikacin isepamicin, dibekacin, arbekacin and tobramycin [1,2]. Aminoglycosides are strong bactericidal antibiotics and their bactericidal action cannot be fully explained through their commonly understood action through inhibition of protein synthesis via irreversible binding to the 30s ribosome. Their bactericidal activity is mainly attributable to their action on bacterial cell membranes [3]. Aminoglycocides being potent antibiotic molecules disrupt the outer cell membrane causing leakage of intracellular contents and eventually bacterial cell death [4].

Aminoglycosides are often recommended for the treatment of life-threatening infections by G-ve bacteria including complicated skin, bone or soft tissue infections, urinary tract infection (UTI), septicaemia, peritonitis and other severe intra-abdominal infections, severe pelvic inflammatory disease, endocarditis, mycobacterium infection, neonatal sepsis, and eye and ear infections [1]. In earlier clinical studies gentamicin has been reported to be a little superior to amikacin, in the treatment of enteric infection, bacteraemia, urinary tract infections, pneumonia and serious soft tissue infections yielding favourable outcomes in 78% and 77% cases, respectively [5]. Evidence suggests that there is no appreciable difference in their nephrotoxic and ototoxic effects [5, 6]. In a early small study on 30

human UTI patients, gentamicin and amicacin had no appreciable difference in the therapeutic outcome [7]. Another study on 1000 bacterial isolates reported better antimicrobial action of gentamicin than amikacin against enterobacteria, *Haemophilus influenzae* and *Staphylococcus aureus* while amikacin was more active against *Klebsiella* and *Providencia* species isolates [8].

In animals, use of aminoglycosides is not recommended unless required for life-saving purposes and systemic use of amikacin and other aminoglycoside antibiotics in animals is mainly avoided due to their nephrotoxicity and ototoxicity [9]. However, in cats, gentamicin appeared to cause more histological renal tissue change than amikacin [10]. In an experimental study in guinea pigs on severity of the cochlear damage, it was the maximum due to gentamicin followed by amikacin, streptomycin, and netilmicin, but the difference in ototoxicity was statistically insignificant between different aminoglycosides [11]. In another study on rabbits, of the four aminoglycosides (tobramycin, sisomicin, amikacin and gentamicin) in the therapy of experimental *E. coli* meningitis sisomycin had marginally better bactericidal action than the other three aminoglycosides [12]. Despite all these observations and indications, aminoglycosides are extensively used in veterinary medicine in treatment of for treatment of bacteraemia, gastrointestinal tract infections, and respiratory and urinary tract infections in many animal species [13]. However, the use of aminoglycosides in animals is not recommended [14] without antimicrobial susceptibility testing (AST) but is almost impracticable in most of developing and poor countries where AST is sparsely available for humans. Though systemic use is rare in food animals in horses and other companion animals aminoglycosides (amikacin and gentamicin) are commonly used to treat septicaemia, respiratory tract infection, peritonitis, metritis, osteomyelitis, leptospirosis, nocardiosis, meningitis, wound infections, joint infections, endometritis, and UTIs caused by ESBL *E. coli* [14]. Although aminoglycosides have no synergy with β-lactam antibiotics [15], they are rarely used alone and mostly prescribed in combination with β-lactam antibiotics [14]. Aminoglycosides as topical use preparation of gentamicin, neomycin and framycetin are often used as drug of choice for the treatment of eye, ear and skin infections in animals and humans [14,16]. Though amikacin and gentamicin belong to the same class of aminoglycosides, it has been suggested earlier that there is no cross-resistance for the two drugs [17]. However, resistance to streptomycin was much higher than resistance to gentamicin in most of the common pathogens affecting animals in the EU. About 2% of the *Enterococcus* spp., *Salmonella enterica*, and *E. coli* isolates from animal cases in the EU are reported resistant to gentamicin and the resistance levels were higher in isolates from conventional broilers [14]. *Enterococcus faecium* and *E. faecalis* isolates were rarely resistant to gentamicin [16]. With the rampant use of aminoglycosides in humans and animals, there is an imminent risk of the emergence of zoonotic pathogens either due to clonal selection of resistant *Mycobacterium tuberculosis*, Enterobacteriaceae members and *Enterococcus* spp., or through horizontal transfer of resistance (R) factors at high frequency among members of Enterobacteriaceae, *E. faecium* and *E. faecalis* [14].

To determine the susceptibility of bacteria to aminoglycosides measurement of minimum inhibitory concentration is the method of choice but in most of the clinical laboratories disk diffusion assays are the method of choice and breakpoints for amikacin applicable to *E. coli* and *P. aeruginosa* isolates from dogs, foals, adult horses, *Staphylococcus* spp. isolates from dogs, *S. aureus* isolates from foals and adult horses, *Streptococcus* spp. isolates from dogs, *Streptococcus equi* subsp*. zooepidemicus* and subsp. *equi* isolates from foals and adult horses are available for long but results may be misleading [18]. *In vitro* tests may indicate the susceptibility of *E. faecalis, E. faecium, E. gallinarum, E. casseliflavus* to aminoglycosides but are rarely of therapeutic utility, and as per CLSI guidelines they should not be reported as susceptible to aminoglycosides [18]. Gentamicin and other aminoglycosides' resistance has more commonly been reported from many countries in *E. coli*, and *Staphylococcus* species especially methicillin-resistant (MRS) isolates from human clinical cases than those isolated from dairy and other domestic animals [14]. Therefore, in the present analytical study, we attempted to understand gentamicin and amikacin susceptibility patterns among bacterial isolates from clinical samples of humans, animals, birds causing infection and from their environment.

MATERIALS AND METHODS

Microbial strains in the study

For the study antimicrobial susceptibility tests data of 517 isolates with known sources and with defined ailments and tested for amikacin, gentamicin, carbapenem susceptibility as per CLSI criteria [18], and extended-spectrum β-lactamase (ESBL) production was retrieved from the Division of Epidemiology Data resources for the last four years (2019-2022). The repository data on bacterial isolates included only those strains which were isolated and identified (Table 1) through conventional methods and confirmed either through specific polymerase reaction, gene sequencing MALDITOF-MS or both.

Detection of extended-spectrum β-lactamase (ESBL) production by Gram-negative bacterial strains

For this purpose E-test was performed using E-strips (Biomeriux India Ltd.) carrying two gradients of ceftazidine and cefotaxime with and without clavulanic acid on Mueller Hinton agar plates as per the recommendations of the E-strip producer [19,20].

Carbapenems, gentamicin (30 µg) and amikacin (30 µg) susceptibility assay

The disk diffusion assay tests were performed and interpreted as per CLSI [18] guidelines. The disks of imipenem (10 µg), meropenem (10 µg) and ertapenem (5 µg) were used for determining susceptibility to carbapenems, bacteria resistant to any of the three carbapenems was considered carbapenem-resistant (CR). All antimicrobial disks and media used in the study were procured from Difco-BBL (USA).

Table 1: Amicacin (Ak), gentamicin (G), carbapenems (Imipenem, meropenem, Ertapenem, IME) and extended spectrum β-lactam antibiotics (cefalosporins) in bacteria isolated from different clinical and environmental samples.

Bacterial species and isolates included in the study	Isolates tested	Resistant to			
		ESBL	IME	G	Ak
Acinetobacter (*A. calcoaceticus baumannii complex* 6, *A. lwoffii* 2, *A. schindleri* 2)	10	0	5	3	2
Aerococcus (*A. sanguinicola* 2)	2	0	0	0	0
Aeromonas (*A. bestiarum* 1, *A. caviae* 1, *A. popoffii* 1, *A. salmonicida* 2, *A. schubertii* 1, *A. sobria* 1)	7	3	1	1	1
Aggregatibacter actinomycetemcomitans 1	1	0	0	0	0
Alcaligenes (*A. denitrificans* 6, *A. faecalis* 7)	13	6	7	2	3
Bacillus (*B. cereus* 2, *B. mycoides* 1, *B. subtilis* 7)	10	0	0	1	0
Bordetella avium 10	10	0	0	0	0
Brevibacillus laterosporus 2	2	0	0	0	0
Brucella abortus 3	3	2	0	1	0
Burkholderia cepacia 3	3	0	2	0	1
Chrysomonas luteola 1	1	0	1	0	1
Citrobacter freundii 6	6	3	0	0	2
Cronobacter sakazaki 2	2	0	0	1	1
Edwardsiella hoshiniae 1	1	0	0	0	0
Enterobacter (*E. cloacae* 1, *E. taylorae* 3)	4	0	0	0	1
Enterococcus (*E. casseliflavus* 2, *E. faecalis* 7, *E. faecium* 15)	24	0	7	9	9
Erwinia mallotivora 1	1	0	0	0	0
Escherichia (*E. coli* 126, *E. fegusonii* 2, *E. hermanii* 1)	129	20	9	35	31
Ewingella Americana 1	1	0	0	0	0
Hafnia alvei 2	2	0	0	0	0
Klebsiella (*K. oxytoca* 4, *K. pneumoniae* 15)	19	6	1	6	4
Kluyvera ascorbata 1	1	0	0	0	0
Koserella trabulsii 1	1	0	0	0	0
Lysinibacillus sphaericus 9	9	0	0	0	1
Micrococcus luteus 1	1	0	0	0	0
Moraxella (*M. catarrhalis* 1, *M. osloensis* 1)	2	1	1	1	1
Morganella morganii 1	1	0	1	1	1
Paenibacillus (*P. amylolyticus* 1, *P. larvae* 1)	2	0	0	0	0
Pantoea agglomerans 29	29	7	2	7	9
Pasteurella (*P. canis* 1, *P. multocida* 2)	3	1	0	0	1
Pectobacterium cyperipedii 4	4	1	0	0	2
Proteus (*P. mirabilis* 12, *P. penneri* 1, *P. vulgaris* 1)	14	3	8	4	11
Pseudomonas (*P. aeruginosa* 15, *P. pseudolacaligenes* 6, *P. stutzeri* 1, *P. testosteronii* 4)	26	4	7	4	7
Raoultella terrigena 3	3	0	2	1	1
Salmonella enterica ssp. enterica 12	12	0	1	2	2
Serratia (*S. entemophila* 1, *S. marcescens* 1, *S. odorifera* 1, *S. plymuthica* 1, *S. rubideae* 1)	5	0	0	1	0
Staphylococcus (*S. arlettae* 1, *S. aureus* 11, *S. capitis ssp. capitis* 6, *S. capitis ssp. urealyticus* 4, *S. carnosus* 1, *S. caseolyticus* 2, *S. chromogenes* 10, *S. cohnii ssp. cohnii* 4, *S. cohnii ssp. urealyticus* 1, *S. delphini* 1, *S. epidermidis* 10, *S. felis* 7, *S. haemolyticus* 19, *S. hyicus* 5, *S. intermedius* 9, *S. lentus* 1, *S. lugdunensis* 3, *S. sacchrolyticus* 1, *S. saprophyticus* 1, *S. schleiferi* 3, *S. warneri* 1)	101	0	6	19	25
Streptococcus (*S. agalactiae* 1, *S. bovis* 1, *S. dysgalactiae* 1, *S. milleri* 13, *S. mitior* 1, *S. pneumoniae* 2, *S. porcinus* 6, *S. pyogenes* 23, *S. suis* 1)	49	0	18	17	22
Vribrio alginolyticus 1	1	0	0	0	0
Xenorhabdus (*X. bovienii* 1, *X. poinarii* 1)	2	1	0	1	0

Types of bacteria					
Gram-positive	200	0	31	46	57
Gram-negative	317	59	48	71	82
Oxidase-positive	80	18	17	9	14
Oxidase-negative	437	41	62	108	125
Oxidase-negative Gram-negative	248	41	31	62	69
Oxidase-negative Gram-positive	189	0	31	46	56
Oxidase-positive Gram-negative	69	18	17	9	13
Oxidase-positive Gram-positive	11	0	0	0	1
Source of bacteia	**Strains**	**ESBL**	**IME**	**G**	**Ak**
Clinical	453	49	64	102	116
Environment (Surface drag swabs 4, drinking water 5, BAS machine scanner swabs 26, Holy basil leaves 4, marketed urine 1, milk 9; pond water 4)	53	9	10	14	19
Reference	11	1	5	1	4
Buffaloes	15	2	1	4	5
Cattle	62	13	8	3	11
Deer (spotted deer 12, Chinkara 1)	13	1	0	1	1
Dogs	86	8	15	25	19
Elephants	4	2	1	0	0
Goats	2	0	0	0	0
Hamster	1	0	0	0	1
Horses	52	4	6	10	14
Humans	85	11	12	22	24
Lions	22	1	3	10	0
Mithuns	14	0	0	10	10
Monkeys	2	0	2	1	2
Mules	4	0	0	0	0
Pigs	23	0	5	1	8
Pigeon	1	0	1	1	1
Poultry birds	43	0	5	6	12
Sanctuary birds (Crane 12, Peacock 6)	18	7	5	5	4
Swamp Buffalo	6	0	0	3	4
Strains of bacteria associated with	**Strains**	**ESBL**	**IME**	**G**	**Ak**
Abortions (7 cattle, 2 buffaloes; A. bestiarum 1, A. schubertii 1, Aggregatibacter actinomycetemcomitans 1, Brucella abortus 3, E. coli 1, Pantoea agglomerans 1, Proteus mirabilis 1)	9	7	1	2	4
Abscess, wounds and other pyogenic infections (1 Buffalo, 5 cattle, 12 deer, dog 19, horse 16, 6 human, 4 mules)	77	6	5	15	18
Ear infections (3 dogs, 4 elephants, 2 humans)	11	2	1	0	0
Eye infections (5 dogs, 6 swamp buffaloes)	11	0	0	5	5
Gastrointestinal tract infections (2 cattle, 11 dogs, 4 humans, 2 monkeys, 12 pigs, 4 poultry birds)	35	1	11	9	13
Genital tract infections (6 cattle, 3 dogs, 4 horses, 14 mithuns)	27	3	1	12	14
Mastitis (2 buffaloes, 31 cattle, 2 goats)	35	7	7	3	6
Pyrexia (7 cattle, 2 horse, 9 humans)	18	0	2	2	3
Respiratory tract infections (1 buffalo, 2 dogs, 4 horse, 21 humans)	28	4	5	3	6
Septicemic deaths (9 buffaloes, 1 cattle, 1 deer, 3 dogs, 1 hamster, 17 horses, 1 human, 22 lions, 11 pigs, 1 pigeon, 39 poultry birds, 18 sanctuary birds)	124	9	16	24	25
Urinary tract infections (3 cattle, 25 dogs, 8 horses, 42 humans)	78	10	15	27	22

Statistical analysis

Data of all 517 strains included in the study along with their source of isolation, association with specified ailment, and susceptibility to amikacin, gentamicin, carbapenems and ESBL production ability was line-entered in an Excel sheet and analyzed using Chi-square statistics to understand the significance of the different associations. For analysis, only those sets were compared where strain numbers or cases were ≥ 6. To determine the relationship among susceptibility to different antibiotics Pearson correlation was done in MS Excel 2007.

FINDINGS (RESULTS)

The *in-vitro* susceptibility study on 517 isolates of bacteria of different origins and associated with different types of infections revealed that gentamicin inhibited more number of bacterial isolates (77.37%) than amikacin (73.11%). Both the antibiotics failed to inhibit 65 (12.57%) of the isolates. However, of the 378 strains of bacteria susceptible to amikacin 52 (13.76%) were resistant to gentamicin, and of the 400 strains susceptible to gentamicin 74 (18.50%) were resistant to amikacin. Correlation analysis of zones of bacterial growth inhibition produced by amikacin and gentamicin revealed a strong (p, <0.001) correlation (r, 0.45) in their antimicrobial activity. Paired t-test analysis of the zone of inhibition by amikacin and gentamicin revealed acceptance of the null hypothesis that there is no significant difference between the susceptibility of microbes to gentamicin and amikacin. A significant relationship (p <0.001) between carbapenem resistance and amikacin (r, 0.264) and gentamicin (r, 0.31) resistance was evident.

Susceptibility to gentamicin and amikacin varied among different bacteria (Table 1) viz., *Alcaligenes* spp. (15.38%, 23.08%), *Enterococcus* spp. (37.50%, 37.50%), *Escherichia* spp. (27.13%, 24.03%), *Klebsiella* spp. (1.58%, 21.05%), *Pantoea agglomerans* (24.14%, 31.03%), *Proteus* spp. (28.57%, 78.57%), *Pseudomonas* spp. (15.38%, 26.92%), *Salmonella enterica* ssp. *enterica* serovars (16.67%, 16.67%), *Staphylococcus* spp. (18.81%, 24.75%), and *Streptococcus* spp. (34.69%, 44.90%), respectively were resistant to gentamicin and amikacin. About 13.33% and 46.67% of *E. coli* isolates from poultry birds were resistant to gentamicin and amikacin, respectively but none of the *E. coli* isolated from infections in pigs was resistant to gentamicin or amikacin.

Though there was no significant difference in susceptibility to amikacin and gentamicin for bacterial strains of different species and causing different ailments, it was evident that where G+ve bacteria were the cause of infection, and isolates from bacterial infections of cattle and pigs significantly (p, <0.025) more number of isolates were susceptible to gentamicin than to amikacin. However, on bacterial isolates from clinical samples from sick lions, amikacin was the better (p, <0.001) antibiotic than gentamicin. Of the 22 isolates of bacteria from infections in lions (*A. calcoacetus-baumanii* complex 1, *Enterobacter cloacae* 1, *Enterococcus casseliflavus* 2, *E. coli* 16, *P. agglomerans* 1, *P. vulgaris* 1), 10 were resistant to gentamicin (*A. calcoaceticus-baumanii* 1, *E. coli* 8, *P. vulgaris* 1) were resistant to gentamicin and none of the isolates was amikacin-resistant. Of the 19 strains of *Klebsiella* species, six were resistant to gentamicin and only four to amikacin. Similarly of the 129 isolates of *E. coli* 35 and 31 were resistant to gentamicin and amikacin, respectively.

Higher proportions of carbapenem-resistant bacteria were (p, <0.001) resistant to gentamicin and amikacin but no significant association (p, 0.98) was apparent with respect to their ESBL production ability. Though for most of the bacteria, ESBL production and susceptibility to gentamicin or amikacin were not significantly (p, >0.05) associated, ESBL *E. coli* were significantly (p, 0.004) more often susceptible to gentamicin (but not to amikacin) than non-ESBL *E. coli*. Oxidase-positive bacteria were significantly more susceptible to amikacin (p, 0.04) and gentamicin (p, 0.01), than oxidase-negative bacteria. However, no such difference was evident with respect to ESBL production and CR. The bacterial isolates from environmental samples were more often (p, 0.04) producers of ESBL than those isolated from clinical sample.

Bacterial isolates from clinical infections in lions were significantly more susceptible to amikacin than those infected buffaloes, cattle, dogs, deer, pigs, birds, mithuns and swamp buffaloes. On the other hand significantly (p, <0.05) more of the bacterial isolates causing infections in mithuns and swamp buffaloes were amikacin-resistant than those infecting other animals. Among all, isolates of *Bacillus* spp. and *Bordetella avium* were the most susceptible to amikacin and *Proteus* spp. strains were often resistant to amikacin. Significantly (p, <0.05) higher proportion of the isolates from genital tract infections (not abortions) were resistant to amikacin than isolates associated with other infections (Table 2).

More often (p, <0.05) bacteria causing genital tract, urinary tract and eye infections were more resistant to gentamicin than those associated with other infections. Among all, *B. avium* isolates were the most susceptible to gentamicin (similar to amikacin) and *Enterococcus* species strains were the most often gentamicin-resistant ones (Table 2).

Carbapenem resistance was more common among bacterial isolates causing gastrointestinal tract ailments followed by those associated with UTIs. Among all, isolates belonging to *Acinetobacter, Alcaligenes, Enterococcus, Proteus, Pseudomonas* and *Streptococcus* species were often resistant to one or more carbapenem antibiotics than *B. avium, Bacillus, Citrobacter, Escherichia, Klebsiella, Lysinibacillus sphaericus, Pantoea agglomerans, Salmonella,* and *Staphylococcus* species strains (Table 2)

Bacteria isolated from clinical cases in deer and mithuns were more often ESBL producers than those associated with infections in other animals. Bacterial isolates from abortion and mastitis were among the most common pathogens having ESBL production ability. More number of bacterial isolates belonging to *Aeromonas, Alcaligenes, Citrobacter* and *Klebsiella* species produced ESBL and only a few of the *Acinetobacter* spp.,

Table 2: Comparative susceptibility of different types of bacteria from various sources to amikacin and other antimicrobials

Antibiotic	Significantly (p, <0.05) more resistant isolates from (of)	Than isolates from (of)
Amikacin	Oxidase- negative	Oxidase-positive
	Buffaloes	Lions
	Cattle	Lions
	Dogs	Lions
	Horses	Lions
	Humans	Lions
	Mithuns	Cattle, deer, dogs, horses, humans, lions, pigs, poultry birds, sanctuary birds,
	Pigs	Lions
	Poultry birds	Lions
	Sanctuary birds	Lions
	Swamp buffaloes	Cattle, deer, dogs, horses, humans, lions, sanctuary birds
	Eye infections	Abscess and wounds, ear infections, septicemic deaths
	Gastrointestinal infections	Ear infections, septicemic deaths
	Genital tract infections	Abscess and wounds, ear infections, mastitis, pyrexia, respiratory tract infections, septicemic deaths, urinary tract infections
	Urinary tract infections	Ear infections
	Citrobacter	*Bacillus, Bordetella avium*
	Enterococcus	*Bacillus, Bordetella avium*
	Pantoea agglomerans	*Bacillus, Bordetella avium*
	Proteus	*Acinetobacter, Aeromonas, Alcaligenes, Bacillus, Bordetella avium, Citrobacter, Enteroccus, Escherichia, Klebsiella, Lysinibacillus sphaericus, Pantoea agglomerans, Pseudomonas, Salmonella enterica* ssp. *enterica, Staphylococcus, Streptococcus*
	Streptococcus	*Bacillus, Bordetella avium, Escherichia, Staphylococcus*
Gentamicin	Oxidase negative	Oxidase positive
	Buffaloes	Cattle, pig
	Dogs	Cattle, pigs
	Horses	Cattle
	Humans	Cattle, pigs
	Lions	Cattle, horses, pigs, poultry birds
	Mithuns	Buffaloes, cattle, dogs, horses, humans, pigs
	Sanctuary birds	Cattle, pigs
	Swamp buffaloes	Cattle, pigs, poultry birds
	Eye infections	Abscess and wounds, ear infections, mastitis, pyrexia, respiratory tract infections, septicemic deaths
	Genital tract infections	Abscess and wounds, ear infections, mastitis, pyrexia, respiratory tract infections, septicemic deaths
	Urinary tract infections	Abscess and wounds, ear infections, mastitis, pyrexia, respiratory tract infections, septicemic deaths
	Enterococcus	*Bordetella avium, Lysinibacillus sphaericus, Staphylococcus*
	Klebsiella	*Bordetella avium*
	Streptococcus	*Bordetella avium, Staphylococcus*
Carbapenem-resistance	Sanctuary birds	Deer, mithuns
	Gastrointestinal infections	Abscess and wounds, eye infections, genital tract infections, mastitis, septicemic deaths,
	Mastitis	Abscess and wounds,
	Urinary tract infections	Abscess and wounds, genital tract infections
	Acinetobacter	*Bacillus, Bordetella avium, Citrobacter, Escherichia, Klebsiella, Lysinibacillus sphaericus, Pantoea agglomerans, Salmonella, Staphylococcus*
	Alcaligenes	*Bacillus, Bordetella avium, Citrobacter, Escherichia, Klebsiella, Lysinibacillus sphaericus, Pantoea agglomerans, Salmonella, Staphylococcus*
	Enterococcus	*Escherichia, Klebsiella, Pantoea agglomerans, Salmonella, Staphylococcus*
	Proteus	*Bacillus, Bordetella avium, Citrobacter, Escherichia, Klebsiella, Lysinibacillus sphaericus, Pantoea agglomerans, Salmonella, Staphylococcus*
	Pseudomonas,	*Escherichia, Pantoea agglomerans, Staphylococcus*
	Streptococcus	*Bacillus, Bordetella avium, Escherichia, Klebsiella, Lysinibacillus, Pantoea agglomerans, Staphylococcus*
Extended-spectrum β-lactamase production	Environmental	Clinical
	Buffaloes	Mithuns, pigs, poultry
	Cattle	Dogs, horses, lions, mithuns, pigs, poultry
	Dear	Dogs, horses, lions, mithuns, pigs, poultry, swamp buffaloes
	Dogs	Horses, Pigs, poultry birds
	Horses	Poultry birds
	Humans	Mithuns, pigs, poultry
	Sanctuary birds	Dogs, horses, lions, mithuns, pigs, poultry, swap buffaloes
	Abortion	Abscess and wounds, eye infection, gastrointestinal infections, genital tract infections, pyrexia, RTIs, septicemic deaths, urinary tract infections
	Ear infection	Eye infections, gastrointestinal infections, septicemic deaths
	Mastitis	Abscess and wounds, eye infections, gastrointestinal infections, genital tract infection, septicemic deaths
	RTI infections	Gastrointestinal infections
	Urinary tract infections	Gastrointestinal infections, septicemic deaths
	Aeromonas	*Acinetobacter, Bordetella avium, Salmonella enterica* ssp. *enterica*
	Alcaligenes	*Acinetobacter, Bordetella avium, Escherichia, Pseudomonas, Salmonella enterica ssp. enterica*
	Citrobacter	*Acinetobacter, Bordetella avium, Escherichia, Salmonella enterica* ssp. *enterica*
	Klebsiella	*Acinetobacter, Bordetella avium, Salmonella enterica* ssp. *enterica*

Bordetella avium, and *Salmonella enterica* ssp. *enterica* strains produced ESBL (Table 2).

DISCUSSION

Amikacin and gentamicin are two commonly used antibiotics in animals, especially in companion animals in India [21]. The detection of gentamicin and amikacin resistance in 23.97% and 22.60% of the isolates from companion animals (dogs, horses), was comparable to gentamicin and amikacin resistance in bacteria causing infections in human beings, 25.8% and 28.24%, respectively. Though more elaborate studies are required, it may be speculated that some kind of clonal selection may exist in many of the bacteria isolated from animals or humans in the present study that were of zoonotic potential [14]. The occurrence of gentamicin and amikacin resistance in 17.48% and 27.18% of the isolates from dairy (cattle, buffaloes and goats), and 13.95% and 27.18% of the isolates from poultry birds, respectively was a bit lower than in bacterial isolates from companion animals and humans but was much lower than those bacteria isolated from semi-domestic swamp buffaloes and mithuns where 65% and 70% of the bacteria isolated were resistant to gentamicin and amikacin, respectively. However, the resistance to aminoglycosides detected in bacterial isolates in India seems to be much higher than that reported in most of the other countries [14,16], but the data compared seems to be much older from other countries and some recent observations made globally needs to be analysed. But this explanation is contradicted by the facts that earlier studies on humans [1,5], reported the effectiveness of amikacin and gentamicin was 77-78% and is quite comparable to the resistance pattern observed in the present study (71.76 to 74.2%). Therefore, more systematic studies on a comparable number of isolates from humans and different animals are required. The high level of amikacin (70%), and gentamicin (65%), resistance in bacteria isolated from semi-domestic animals is of high concern as these animals may spread AMR pathogens in the environment of a larger geographical area. The AMR traits might be persisting in the semi-domestic animals due to some clonality or something else, needs more elaborate molecular studies on AMR in bacterial isolates from semi-domestic animals, interestingly, despite the high occurrence of aminoglycoside resistance none of the isolates from semi-domestic animals had either carbapenem resistance or produced ESBL. However, the observation of a significantly high occurrence of amikacin and gentamicin resistance in strains of semi-domestic mithuns (*Bos frontalis*) and swamp buffaloes (*Bubalus bubalis kerebau*) and also in wild animals and birds than in isolates of cattle and pig origin could not be substantiated with available literature but aminoglycoside resistance has rampantly reported in bacteria causing lethal infections in zoo and wild animals and birds [22, 23].

The observations revealed that 25.11% and 26.84% of isolates belonging to members of Enterobacteriaceae were resistant to gentamicin and amikacin and observations corroborate earlier studies on a large number of bacteria [8], indicating better but insignificantly different activity of gentamicin than amikacin. However, in contrast to other members of Enterobacteriacea *Klebsiella* species isolates were more susceptible to amikacin (78.95%) than to gentamicin (68.42%) similar to earlier observations [8].

The study indicated that gentamicin was significantly (p, 0.003) more effective on ESBL *E. coli* than on non-ESBL *E. coli* and a similar trend but statistically insignificant was observed for amikacin (>25% non-ESBL *E. coli* and 15% of ESBL *E. coli* were resistant, indicating the utility of aminoglycosides to treat infections caused by ESBL *E. coli.* The observations are in line of observations in many of the EU nations [14].

Of the 24 isolates of enterococci, nine each were resistant to amikacin and gentamicin and seven to carbapenems too. Carbapenem resistance in enterococci is commonly reported despite being susceptible to penicillin and penicillin derivatives due to the presence of variant or overproduced penicillin-binding proteins [24], and carbapenem-resistant enterococci have commonly been reported causing infections in animals and inhabiting their environment [25,26]. Though enterococci are often reported as resistant to aminoglycosides [27], some studies reported susceptibility of *E. faecium* and *E. faecalis* isolates to gentamicin [16]. Enterococci isolates were significantly more often resistant to aminoglycosides than isolates of other bacteria and this may be attributed to intrinsic and acquired resistance in enterococci [24]. A total of 29.17% of the enterococci were carbapenem-resistant. The observations are in concurrence with earlier reports [24,28]. In the study, seven strains were resistant to both gentamicin and amikacin but two strains each were resistant to only one of the two antibiotics. A similar variation in susceptibility to different aminoglycosides in strains of enterococci has commonly been reported [24].

Among all the bacteria tested, *Proteus* strains were the most resistant to amikacin (78.57%), which may be due to fast acquisition of transmissible amikacin resistance by *Proteus* species [29,30]. Though amikacin was suggested to be one of the best antibiotics for treating infections caused by MDR strains of *Proteus* species strains in 20th centry [31], it seems to be useless now. In the present study, gentamicin was significantly more effective on *Proteus* strains inhibiting 71.43% of the isolates than amikacin (21.43%) and observations further confirm the therapeutic utility of gentamicin for infections by *Proteus* species strains [32].

Bacteria causing eye infections and genital tract infections were more often resistant to gentamicin as well as amikacin than bacteria causing, abscess, wound, ear, and respiratory tract infections, and septicaemia; this may be of serious concern as at one time amikacin was considered as gold standard treatment for the treatment of genital tract infections [33], and still now aminoglycoside preparations are often recommended for treatment of ophthalmic and genital tract infection in humans [34,35]. Besides, bacteria causing gastrointestinal infections in animals and birds were more resistant to amikacin than those isolated from cases of ear infections and septicaemia is also

of concern as bacteria present in excreta may contaminate environment and water bodies [26].

CONCLUSIONS, LIMITATIONS AND RECOMMENDATIONS

This analytical study concludes that amikacin and gentamicin resistance is common among bacteria causing infections in animals too despite the fact that therapeutic use of aminoglycosides is restricted in animals and birds. Further, the preferred use of aminoglycosides in the treatment of eye infections and genital tract infections appears to be erroneous as bacteria isolated from eye and genital tract infections were not only more resistant to amikacin but also to gentamicin than bacteria causing other infections. The major limitation of the study is non-equitable numbers of the isolates of different bacteria and of different sources compared for efficacy of the amikacin and gentamicin. Another limitation is, the study analysed *in-vitro* susceptibility data while it is known fact that *in-vivo* or therapeutic outcome sometimes may not match with *in-vitro* observations. The study recommends that looking at the wide variation in the susceptibility of bacterial strains of different species causing various types of infections in animals and birds under different husbandry practices suggests that antimicrobial susceptibility should be conducted before the use of amikacin or gentamicin in therapeutics.

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REFERENCES

1. Gilbert DN. Aminoglycosides. In: Mandell GL, Bennett JE, Dolin R, editors. Douglas and Bennett's principles and practice of infectious diseases. New York: Churchill Livingston, 1995; 279-301.
2. van Hoek AH, Mevius D, Guerra B, Mullany P, Roberts AP, Aarts HJ. Acquired antibiotic resistance genes: an overview. Front Microbiol. 2011; 2: 1-27.
3. Singh BR. Antibiotics: introduction to classification. Divisional seminar at Division of Epidemiology, ICAR-Indian Veterinary Research Institute, Izatnagar-243 122, India. 2015.
4. Montie T, Patamasucon P. Aminoglycosides: the complex problem of antibiotic mechanisms and clinical applications. Eur J Clin Microbiol Infect Dis. 1995; 14: 85-87.
5. Smith CR, Baughman KL, Edwards CQ, Rogers JF, Lietman PS. Controlled comparison of amikacin and gentamicin. N Engl J Med. 1977; 296: 349-353.
6. Alinejad S, Yousefichaijan P, Rezagholizamenjany M, Rafie Y, Kahbazi M, Ali A. Nephrotoxic effect of gentamicin and amikacin in neonates with infection. Nephro-Urol Mon. 2018; 10: e58580.
7. Gilbert DN, Eubanks N, Jackson J. Comparison of amikacin and gentamicin in the treatment of urinary tract infections. The American J Med. 1977; 62: 924-929.
8. Forgan-Smith WR, McSweeney RJ. Gentamicin and amikacin---an in vitro comparison using 1000 clinical isolates. Aust N Z J Med. 1978; 8: 383-386.
9. Singh, BR. Guidelines for antimicrobial drug use in animals. 2020.
10. Christensen EF, Reiffenstein JC, Madissoo H. Comparative ototoxicity of amikacin and gentamicin in cats. Antimicrob Agents Chemother. 1977; 12: 178-184.
11. Kalkandelen S, Lu E, An F, Üçüncü H, Altas E. Comparative cochlear toxicities of streptomycin, gentamicin, amikacin and netilmicin in guinea-pigs. J Int Med Res. 2002; 30: 406-412.
12. Strausbaugh LJ, Mandaleris CD, Sande MA. Comparison of four aminoglycoside antibiotics in the therapy of experimental E. coli meningitis. J Lab Clin Med. 1977; 89: 692-701.
13. EMA/ESVAC, 2017. European Medicines Agency, European surveillance of veterinary antimicrobial consumption. sales of veterinary antimicrobial agents in 30 European countries in 2015 (EMA/184855/2017). Trends from 2010 to 2015. Seventh ESVAC report.
14. Committee for Medicinal Products for Veterinary Use (CVMP). Reflection paper on use of aminoglycosides in animals in the European Union: development of resistance and impact on human and animal health EMA/CVMP/AWP/721118/2014. 2018.
15. Gloyd J. Regulatory front: penicillin/streptomycin combinations to disappear in 1993. J Am Vet Med Ass. 1992; 201: 1826-1832.
16. DSAVA. Antibiotic use guidelines for companion animal practice. 2015.
17. Klastersky J, Odio W, Hensgens C. Comparison of amikacin and gentamicin. Clin Pharmacol Therapeut. 1975; 17: 348-354.
18. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 27th ed. CLSI supplement M100, ISBN 1-56238-804-5, Wayne: Clinical and Laboratory Standards Institute. 2017.
19. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: A clinical update. Clin Microbiol Rev. 2005; 18: 657-686.
20. Rawat D, Nair D. Extended-spectrum β-lactamases in Gram negative bacteria. J Glob Infect Dis. 2010; 2: 263-274.
21. Singh BR. Antimicrobial drug uses by veterinarians in equine clinical cases in India. Res J Vet Sci. 2010; 3: 165-178.
22. Singh BR, Mathesh K, Pawde AM, Karthikeyan R, Sinha DK, Agri H. Antimicrobial Susceptibility profile of bacterial culturome of heart blood samples of big cats died in zoos and wildlife sanctuaries in Northern India. Acta Scientific Microbiol. 2022; 5: 104-115.
23. Singh BR, Agri H, Karthikeyan R, Jayakumar V. Common bacterial causes of septicaemia in animals and birds detected

in heart blood samples of referred cases of mortality in Northern India. J Clin Med Img. 2023; 7: 1-14.

24. Kristich CJ, Rice LB, Arias CA. Enterococcal infection—treatment and antibiotic resistance. In: Gilmore MS, Clewell DB, Ike Y editors. Enterococci: from commensals to leading causes of drug resistant infection. Boston: Massachusetts Eye and Ear Infirmary. 2014.

25. Singh BR. ESKAPE Pathogens in animals and their antimicrobial drug resistance pattern. J Dairy Vet Anim Res. 2018; 7: 10-15.

26. Singh BR, Karthikeyan R, Sinha DK, Vinodh Kumar OR, Jakumar V, Yadav A, et al. potentially pathogenic bacteria in water bodies and drinking water supplies in and around Bareilly, India. Acta Scientific Microbiol. 2022; 5: 113-126.

27. Handwerger S, Raucher B, Altarac D, Monka J, Marchione S, Singh KV, et al. Nosocomial outbreak due to Enterococcus faecium highly resistant to vancomycin, penicillin, and gentamicin. Clin Infect Dis. 1993; 16: 750-755.

28. Metzidie E, Manolis EN, Pournaras S, Sofianou D, Tsakris A. Spread of an unusual penicillin- and imipenem-resistant but ampicillin-susceptible phenotype among Enterococcus faecalis clinical isolates. J Antimicrobial Chemother. 2006; 57: 158-160.

29. Girlich D, Bonnin RA, Dortet L, Naas T. Genetics of acquired antibiotic resistance genes in Proteus spp. Front Microbiol. 2020; 11: 256.

30. Wachino JI, Yamane K, Shibayama K, Kurokawa H, Shibata N, Suzuki S, et al. Novel plasmid-mediated 16S rRNA methylase, RmtC, found in a Proteus mirabilis isolate demonstrating extraordinary high-level resistance against various aminoglycosides. Antimicrob Agents Chemother. 2006; 50: 178-184.

31. Sharp PM, Saenz CA, Martin RR. Amikacin (BB-K8) treatment of multiple-drug resistant Proteus infections. Antimicrob Agents Chemother. 1974; 5: 435-538.

32. Jamil RT, Foris LA, Snowden J. Proteus mirabilis infections. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. 2023.

33. Ledger WJ. Aminoglycosides in gynecologic infections. Am J Med. 1986; 80: 216-221.

34. Thy M, Timsit JF, de Montmollin E. Aminoglycosides for the Treatment of severe infection due to resistant Gram-negative pathogens. Antibiotics. 2023; 12: 860.

35. Towne TG. Aminoglycosides. In: Philip Wexler, Editor. Encyclopedia of Toxicology (3rd Edn). Bethesda: Academic Press. 2014; 191-193.