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Assessment of antibiotic sensitivity patterns of bacterial isolates in dogs with positive urine cultures with and without renal dysfunction and variable urinary tract signs

V. Garud Kaustubh[1], Y. Buduk Shreya[2], A. Deosthali Samruddhi[3], N. Galdhar Chandrakant[2], S. Sole Sushant[4], D.A. Pawalkar[5], V. Gaikwad Rajiv [2].

[1]Department of Veterinary Nuclear Medicine, [2]Department of Clinical Veterinary Medicine, Ethics and Jurisprudence, [3]Undergraduate student, [4]Department of Pharmacology and Toxicology, [5]Department of Preventive Veterinary Medicine and Epidemiology, Mumbai Veterinary College, Parel, Mumbai -12

Abstract

Positive bacterial isolates from urine samples of 42 dogs presented with signs of either urinary tract distress or renal dysfunction were retrospectively studied for the bacteria isolated and their sensitivity patterns. *E-coli* (64.29%), *Pseudomonas aeruginosa* (11.9%), *Klebsiella pneumoniae* (11.9%), *Proteus mirabilis* (7.14%), were the most common bacterial isolates followed by *Staphylococcus aureus* (2.3%) and *Enterococcus fecalis* (2.3%). Clinical bacteriuria was observed in 29% (n=12) patients. Among the patients with underlying renal dysfunction, clinical bacteriuria was observed in 9% (n=4) while majority 62% (n=26) had subclinical bacteriuria. Overall maximum *in-vitro* sensitivity was towards Aminoglycosides namely, Amikacin (88.1%), Gentamicin (76.19%); followed by Nitrofurantoin (61.9%); Fluroquinolones namely, Ciprofloxacin (45.24%) and Penicillins namely, Amoxicillin-clavulanic acid (16.6%) and Ampicillin-Sulbactum (16.6%). Cephalosporins showed varying intra-class sensitivity. Decreasing sensitivity and/or increasing resistance towards commonly prescribed antimicrobials narrows the choice of antimicrobials in routine practice and emphasizes on urinary antibiotic sensitivity profile in guiding treatment protocols for urinary tract infections, more so in patients with underlying renal dysfunction.

Key words: *E-coli,* Clinical bacteriuria, Subclinical bacteriuria, Renal dysfunction, Antibiotic sensitivity, Antibiotic resistance

Presence of bacteria in urine and extent of bacterial load is confirmed by a positive urine culture of aseptically collected urine sample from a patient suspected for urinary tract infection or distress. The antibiotic sensitivity profile of the bacterial isolate is expected to be utilised to guide the antibiotic therapy. There is poor agreement between positive urine analysis and urine culture results, which precludes treatment of urinary tract infections without culture (McGhie *et al*., 2014). Improper or injudicious therapy have been known to cause variety of health concerns in patients as well as offer evolutionary advantage to the untreated or undertreated bacterial population resulting in development of antibiotic resistant bacteria inviting much larger public health concern (Weese *et al*., 2019).

Urinary tract infection (UTI) is defined as adherence, multiplication, and persistence of infectious agent in the urogenital system. Anatomical or functional host resistance normally prevent microbial invasion in the urinary tract. Development of UTI, thus depends upon the balance between infectious agents and host resistance. Urinary tract infection is common in dogs with, reporting an incidence of 10-14 per cent (Hall *et al*., 2013). Studies in veterinary literature described a higher UTI prevalence in dogs with various underlying diseases, including diabetes mellitus (37%), hyperadrenocorticism (46%), thoracolumbar vertebral disk herniation (38%), and obesity (25%). The reported prevalence of UTIs in cats with CKD was 22 and 29 per cent (Mayer-Roenne *et al*., 2007 and White *et al*., 2013). Studies also report that bacteriuria in dogs with kidney disease is as much 30-32 per cent (Wong *et al*., 2015; Lamoureux *et al*., 2019). Among bacterial causes, *Escherichia coli* has been the most frequently isolated bacteria causing UTI in dogs which can go up to 44 per cent (Ball *et al*., 2008; Hall *et al*., 2013). Other commonly isolated bacteria include *Staphylococcus* spp., *Enterococcus* spp., *Proteus* spp., *Pseudomonas* spp., and *Klebsiella* spp.

It is important to note that the bacteriuria could be prevalent in general population without overt clinical signs of urinary tract distress, albeit this number is exceptionally low in healthy population (2.1%) as per observations of McGhie *et al*., (2014). However, with presence of co-morbidities like chronic kidney disease - the number of positive urine cultures in the absence of

Corresponding author: *Kaustubh.garud@gmail.com*

signs of lower urinary tract distress was observed to be about 45 per cent (Foster *et al*., 2018).

Materials and Methods

Positive bacterial isolates from the urine samples of 42 dogs presented with signs of either urinary tract distress or renal dysfunction were retrospectively studied for the bacteria isolated and their sensitivity patterns. The urine samples were aseptically harvested and were transported immediately to NABL (National Accreditation Board for Testing and Calibration Laboratories) accredited laboratory and results received were collated. Bacterial growth >100000 CFU/ml was considered clinically significant. Isolates were classified as Susceptible, Intermediate or Resistant based on published serum breakpoints as per the relevant Clinical Laboratory Standard Institute Guidelines.

Results and Discussion

A) Distribution of bacterial isolates & their antibiotic sensitivity patterns

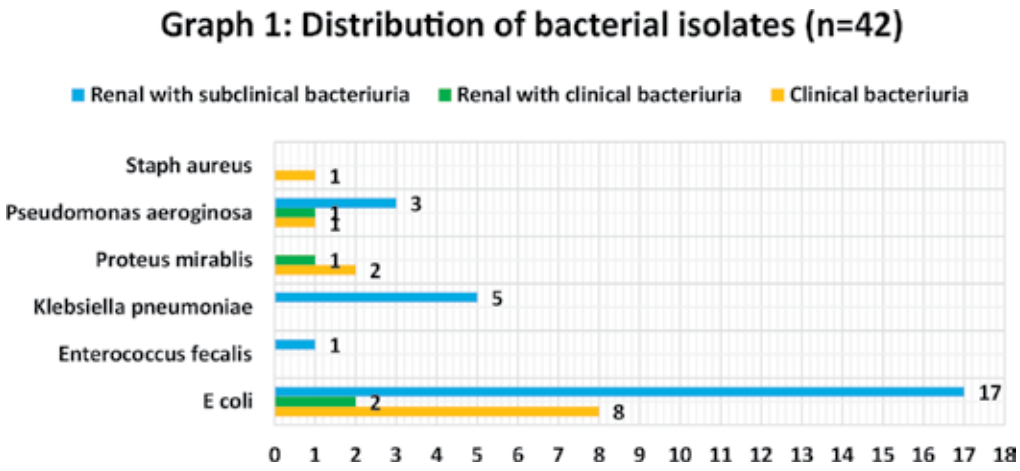
Bacterial isolates were identified from urine samples of 42 dogs. The most common bacterial isolate was *E-coli*, isolated in 64.29 % (n=27), followed by *Pseudomonas aeruginosa* 11.9 % (n=5), *Klebsiella pneumoniae* 11.9 % (n=5) and *Proteus mirabilis* 7.14 % (n=3). *Staphylococcus aureus* and *Enterococcus fecalis* isolates were isolated from 1 urine sample each. The distribution of the different isolates from dogs presented with different clinical complaints is illustrated in Fig.1.

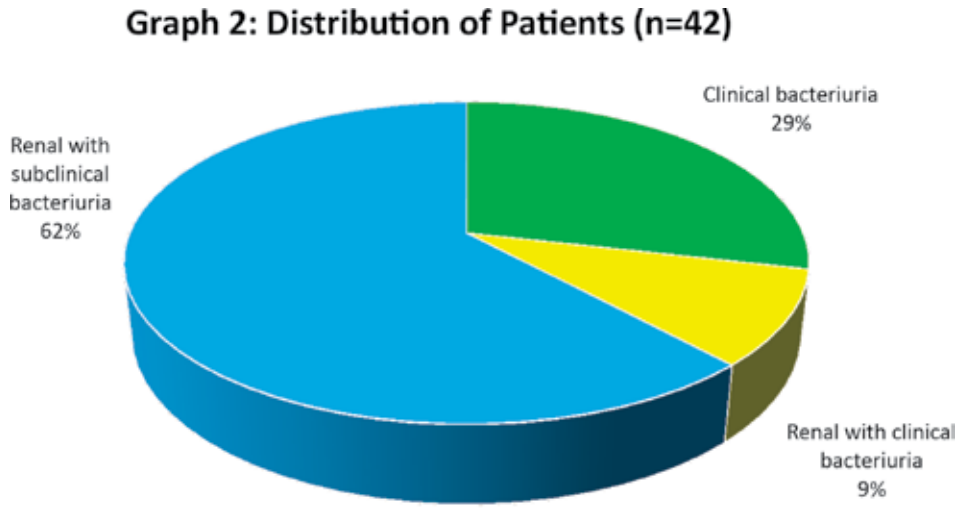
Clinical bacteriuria was observed in 29 % (n=12). Among the patients with underlying renal dysfunction, clinical bacteriuria was identified in 9 % (n=4) while majority 62 % (n=26) were classified as having subclinical bacteriuria. Fig. 2 represents distribution of patients with clinical picture of the bacteriuria.

In vitro susceptibility among all isolates varied for commonly prescribed antimicrobials with highest sensitivity towards Aminoglycosides namely, Amikacin (88.1%), Gentamicin (76.19%); followed by Nitrofurantoin (61.9%); Fluroquinolones namely, Ciprofloxacin (45.24%), Penicillins namely, Amoxicillin-clavulanic acid (16.6%) and Ampicillin-sulbactum (16.6%). Cephalosporins showed varying intra-class sensitivity with highest sensitivity for Cefperazone-sulbactum (54.76%) followed by, Cefepime (47.62%), Cefotaxime and Ceftriaxone (38.1%), Cefexime (33.33%) and Cephalexin (26.19%). Among the commonly used antimicrobials, the overall resistance was least for Amikacin (9.52%). Highest susceptibility was recorded for Carbapenams (95.24%) and Colistin (90.48%) but these drugs are often used as last resort to multidrug resistant bacteria in human medicine and their use in veterinary medicine requires strict adherence to guidelines. (Abraham *et al.*, 2014 and Weese *et al*., 2011). The only isolate of *Staphylococcus* isolate was found to be resistant to Carbapenams while one *Pseudomonas* isolate was intermediate susceptible to Carbapenams. Three isolates of *Proteus* showed resistance to Colistin.

The most common bacterial isolate, *E-coli*, was resistant to most of the antimicrobials like Amoxicillin-clavulanic acid (85.19%), Cefotaxime and Ceftriaxone (59.26%), Amikacin (7.41%) and Gentamicin (14.81%), Ciprofloxacin (51.85%) and Nitrofurantoin (7.41%). These drugs may be being used as first-line antimicrobials with increasing frequency in cases of suspected urinary infection or any other bacterial infection per se, which might have led to the development of resistance. Highest sensitivity was recorded for Carbapenams and Colistin (100%), followed by Amikacin (92.59%) and Gentamicin (81.48%). Fig. 3 illustrates the antibiotic sensitivity patterns for isolates of *E.coli*.

Pseudomonas aeruginosa was second common bacterial isolate which was susceptible to Cefepime (80%), Carabapenams (80%), Colistin (100%), Amikacin (80%), Ciprofloxacin (40%) and Chloramphenicol (40%). *Klebsiella pneumoniae* was isolated from urine samples of 11.9 % (n=5) dogs. All these dogs had underlying renal dysfunction. Maximum sensitivity of *Klebsiella* sp. was observed for Amikacin (100%) and Piperacillin-tazobactum (80%), followed by Cephalosporins, Fluoroquinolones and gentamicin (60%), Nitrofurantoin (40%) and least towards Amoxicillin-clavulanic acid (20%). *Proteus mirabilis* isolate was highly susceptible to Piperacillin-tazobactum, Carabapenams and Amikacin (100%), followed by Amoxicillin-clavulanic acid, Cephalosporins and Fluoroquinolones. Figures 4, 5 and 6 represents antibiotic sensitivity patterns for isolates *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Proteus mirabilis* respectively. Multidrug resistant *Staphylococcus aureus* was isolated which was susceptible to only Vancomycin and Linezolid. *Enterococcus fecalis* isolate was susceptible to most of the Penicillins, Beta lactums, Carbapenams, Nitrofurantoin and Gentamicin, whereas was resistant to Cephalosporins,





Fluoroquinolones and Amikacin.

B) Distribution of bacterial isolates from patients with renal dysfunction and their antibiotic sensitivity patterns

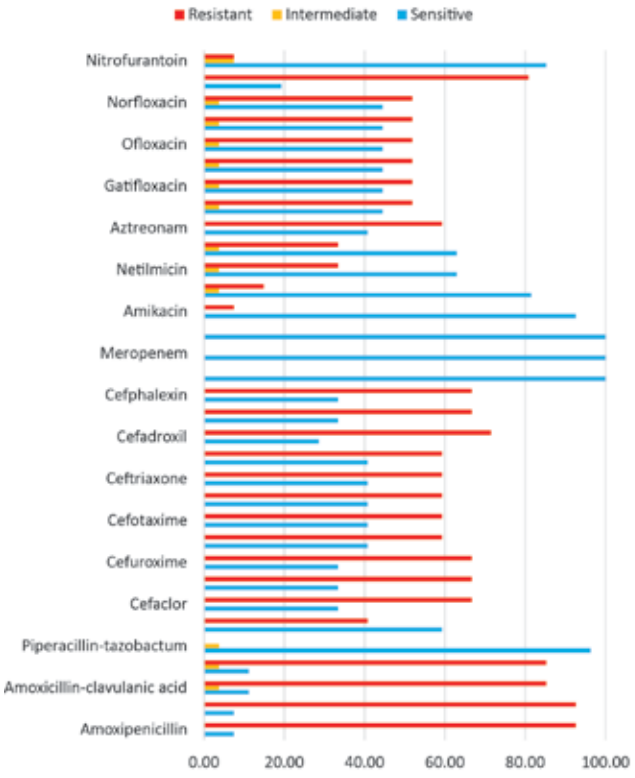
Amongst 42 dogs with significant bacteriuria, 30 dogs (71.42%) had underlying renal disease. Bacterial isolates from urine samples of these dogs also showed a high incidence of *E-coli* organism 64% (n=19/30), followed by *Klebsiella pneumonia* 17%, *Pseudomonas aeruginosa* 13% and *Proteus mirabilis* and *Enterococcus fecalis* 3% each.

Among the patients with renal dysfunction demonstrating clinical bacteriuria, isolated organisms showed highest sensitivity towards Carbapenams and aminoglycosides (100%), followed by Fluoroquinolones (75%), Cephalosporins namely Cefepime (75%), Cefotaxime, Ceftriaxone and Cefixime (50%), Amoxicillin-clavulanic acid (50%) and Nitrofurantoin (50%). Fig. 7 represents the antibiotic sensitivity patterns in patients with renal dysfunction and clinical bacteriuria.

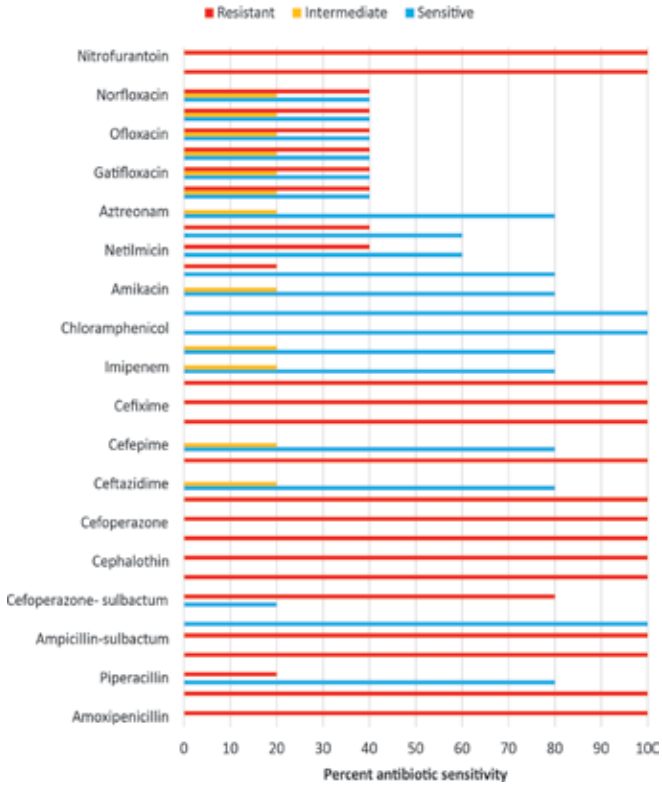
Figure 8 represents the antibiotic sensitivity pattern in dogs with renal dysfunction with subclinical bacteriuria. Bacterial isolates showed highest susceptibility towards Carbapenams (96%) followed by Amikacin (84%), Gentamicin (69%), Nitrofurantoin (69%), third generation Cephalosporins (42%), Fluoroquinolones (38%) and least for Amoxicillin-clavulanic acid (15%).

This study represents evaluation of positive urine cultures in dogs with significant bacteriuria. Among the different bacterial isolates, *E-coli* (64.29%), *Klebsiella* (11.90%) and *Pseudomonas* (11.90%) were the most prevalent, followed by *Proteus mirabilis* (7.14%), *Staphylococcus aureus* (2.38%) and *Enterococcus fecalis* (2.38%). These findings are in conformity with reports by other researchers (Roberts *et al.* (2019), Hall *et al.*

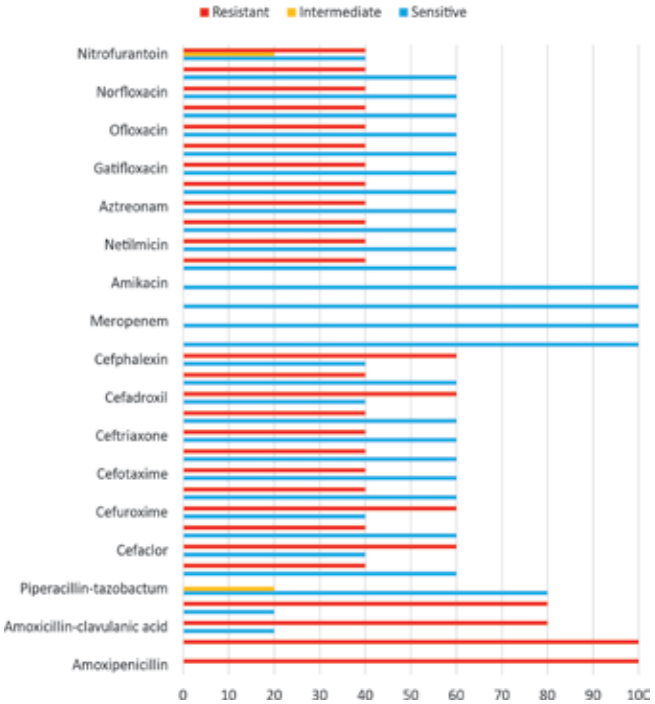
Graph 3: Antibiotic sensitivity pattern of E. coli (n=27)



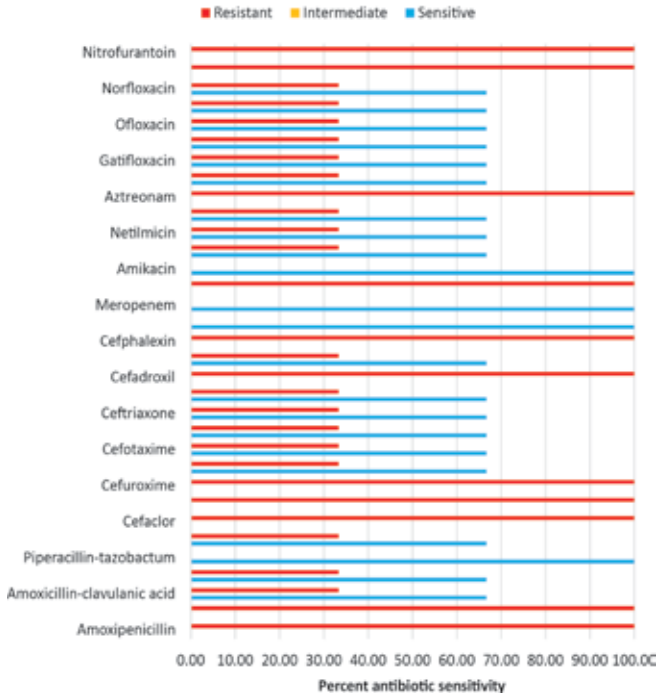
Graph 4: Antibiotic sensitivity pattern of Pseudomonas (n=5)



Graph 5: Antibiotic sensitivity pattern of Klebsiella (n=5)



Graph 6: Antibiotic sensitivity pattern for Proteus mirablis (n=3)



(2013) and Oh *et al.* (2017). Although these studies showed *Staphylococcus* as the second most important bacterial isolate in UTI in dogs, our study observed a low incidence of *Staphylococcus* organism causing UTI. This varied prevalence may be because the study was undertaken on referral cases which have been treated with antibiotics on multiple occasions in past, potentially imparting selection pressure on bacteria in urinary tract. Additionally, geographical differences in microbiodata of animals may have contributed in differences in the prevalence of bacterial isolates. Increased prevalence of antimicrobial resistant populations in canine urinary tract has been an upcoming clinical and public health concern. The resistance is, in particularly for Fluoroquinolones, third generation Cephalosporins, and Clavulanic acid-potentiated Beta-lactams, which are all regarded as second-line veterinary antimicrobial agents (Thompson *et al*., 2011). The reasons for emerging resistance to second line antimicrobials lies in years of injudicious and indiscriminate use of these antibiotics in general veterinary practice.

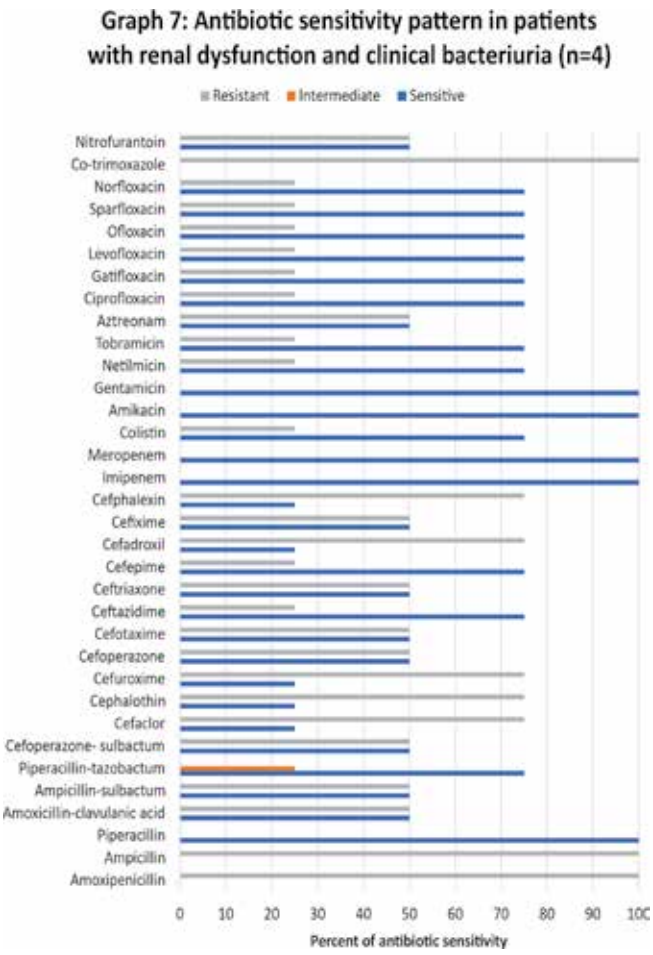
Our study demonstrated a similar trend, with a high resistance of bacterial isolates towards Amoxicillin-Clavulaunic acid and Ampicillin-Sulbactum (80.95%), Cefotaxime and Ceftriaxone (61.9%) and Ciprofloxacin (50%). Interestingly, the Cephalosporins showed a varying intra-class resistance pattern. First generation (Cephalexin) and second generation (Cefachlor and Cefuroxime) showed highest resistance (73.81%) towards bacterial isolates. Third generation (Cefperazone, Cefotaxime and Ceftriaxone) showed a resistance of 61.90% while Cefexime showed a resistance of 66.67%. Fourth generation Cefepime showed a resistance of 50%. Therefore, prescription of antimicrobial drug should be based on maximum sensitivity of the individual antimicrobial and should not be adjudged for the entire class as a whole.

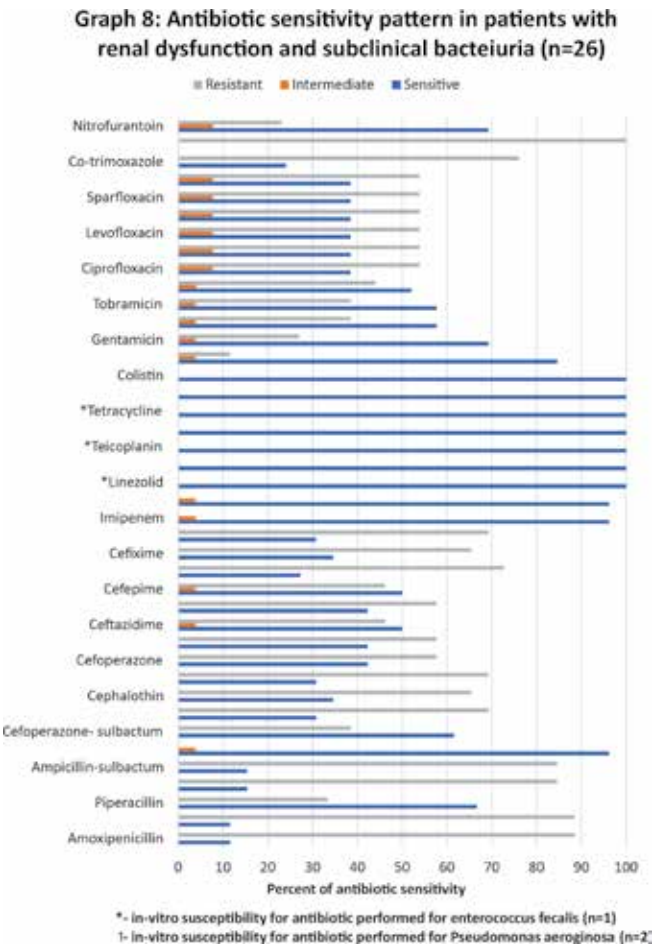
Antimicrobial resistance in *E-coli* has increased worldwide and its susceptibility patterns show substantial geographic variation, possibly due to influence of locally favourite antimicrobials used in general practice. A study of canine urinary isolates over 9 years (1992–2001) demonstrated increasing resistance to Fluoroquinolones over time, particularly for *E-coli* (Cohn *et al*., 2003). In this study, the overall resistance of *E-coli* to antimicrobials was high and was observed to be consistent with the findings of earlier studies by different authors. The resistance towards Penicillins show similar trends but is higher for Cephalosporins and Fluoroquinolones and lower for Aminoglycosides when compared with the findings of Qekwana *et al*. (2018).

Amongst all our 42 patients, 26 patients (62%) were observed to have subclinical bacteriuria. All these patients were azotemic and their urine sample was harvested as a part of their evaluation for renal dysfunction. Amongst all renal patients (n=30), 26 (86.6%) has subclinical bacteriuria. This was higher than Foster *et al.* (2018) who recorded 45% incidence of subclinical bacteriuria in dogs with CKD. This is likely to be due to relatively small sample size (42 versus 182 patients) and suspected overrepresentation. Only 9% dogs with renal dysfunction showed symptomatic bacteriuria which was in accordance with Lamoureux *et al.* (2019) who documented signs of urinary tract disease in 8% of dogs with positive urine culture and CKD. A high incidence of subclinical bacteriuria in dogs with renal dysfunction might be due to the lowered local host defences like reduced renal defences, alterations in urine pH (high or low) and warrants further research (Senior, 2011). *E-coli* was the major pathogen isolated amongst renal patients with prevalence of 63.33%. The recorded incidence in the present study was slightly lower than those observed by Foster *et al.* (2018) and Lamoureux *et al.* (2019) who isolated *E-coli* in 76% and 71% of dogs with CKD respectively. The plausible reason for the difference in observations could be due to the mixed population of acute, acute on chronic and chronic kidney disease patients in our study.

It is important to note that, the involvement of renal dysfunction as a co-morbid factor precludes use of potentially nephrotoxic antimicrobials for treatment of urinary tract bacteriuria, at the same time may put the clinician in obligation of treating the demonstrated bacteriuria so as to prevent future episodes of ascendance of bacteria in upper urinary tract and complicate the patient's condition and influence prognosis negatively. The emergence of multi-drug resistant pathogens in urinary tract of patients with renal disease substantially narrows the antimicrobial choices.

Although, the treatment of subclinical bacteriuria in patient with renal disease or established UTI in otherwise healthy patient is based on achieving adequate antibiotic urinary concentrations in relation to the susceptibility of the offending pathogen, this only represents a minor piece of the puzzle. As most of the antimicrobials are cleared via kidneys, it is imperative that the excretion





of antibiotics through a compromised kidney would be affected resulting in altered bioavailability of antibiotic in urinary tract. It would also be of paramount importance that the antibiotics are selected wisely after appropriate adjustment in dosage of dosing interval to minimize its accumulation in body and potential toxicity, when used in patients with compromised renal clearance.

The rate and extent of renal elimination of Aminoglycosides and Sulphonamides correlate with renal function, as they are solely excreted by glomerular filtration. In contrast, Penicillins, Cephalosporins, and Fluoroquinolones are eliminated through both glomerular filtration and tubular secretion, allowing for high urinary concentrations (Chastain *et al*. 2018). Shimizu *et al*. (2017) clarified the extremely high urinary concentration of Fluoroquinolones like Orbifloxacin, Enrofloxacin and Ciprofloxacin, which was over 100 times higher than the maximum drug concentration in plasma after oral administration at the same dose in healthy individuals. They also mentioned that once daily dosing of Fluoroquinolones maintains a high urinary concentration up to 24 hrs. which is essential for a drug to be effective for treating UTI.

In the present study, although a high susceptibility was recorded for Aminoglycosides like Amikacin (86.67%) and Gentamicin (73.33%), these drugs being nephrotoxic, limit their administration in patients with renal disease. High susceptibility was recorded for Carbapenams (96.67%) and Colistin (96.67%), but their potential nephrotoxic effect in addition to top shelf nature of these drugs limits their use in routine clinical practice. Amoxicillin is a good first line option drug for uncomplicated and complicated UTIs (Weese *et al.* 2011), however, a resistance in 80% of isolates was seen for Amoxicillin-clavulanic acid. In the present study, dogs with renal dysfunction showed a high resistance towards Cephalosporins (40-70%) and Fluoroquinolones (50%), which is comparable with the observations of Cohn *et al*. (2003). This tends to out-date the conventional notion of "antimicrobials with anticipated effectiveness" and make it imperative that the most effective and renal safe (wherever applicable) antibiotic is selected based on

susceptibility patterns.

Ongoing injudicious and indiscriminate use of antimicrobials persistently exerts evolutionary pressure on bacteria to triumph and become resistant to exposed antimicrobials, making it imperative not only to select antimicrobials based on the sensitivity patterns but also to keep a close vigil on achieved bacteriostatic or bactericidal action in vivo. This becomes a clinical necessity when patients involved have compromised renal clearance. It is, therefore, prudent to realize that although a bacterium is sensitive to a specific antibiotic in vitro, it may not translate in vivo due to numerous factors such as altered drug metabolism or excretion, more so in patients with renal or hepatic dysfunction. Minimum Urinary Concentration (MUC) of an antibiotic is hence an important parameter for clinical success of a treatment for urinary tract infection. However, more research to gather more clinical information involving urinary concentrations of antibiotics is required.

Conflicts of interest: The authors declare that they have no competing interests.

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