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Utility of portable magnetic nanoparticle based kit for selection of antibiotics in therapeutic management of bacterial infection of animals

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Abstract
CanAntibiotic Quick Select Kit is an in *vitro* diagnostic assay that helps to select the correct antibiotic for clinical application in canines. The present study was undertaken in healthy as wells as diseased 25 clinical samples of dog and other species brought to Veterinary Clinical Complex, Namakkal during the year 2015 to select the appropriate antibiotic to treat the clinical conditions using the rapid field test kit. Twenty-five clinical samples collected respectively from dog (15), cattle (5), sheep (3) and poultry (2) were tested using the kit. The samples were tested against eight antibiotics viz., ciprofloxacin, ceftriaxone, ceftriaxone and tazobactum combination, ceftiofur, enrofloxacin, ampicillin and potassium clavulanate combination, ampicillin and dicloxacillin combination and finally amoxicillin, cloxacillin combination. Surprisingly, out of the twenty-five samples tested, 80 percent (12/15) from dog and 60 percent (3/5) from cattle were found to be resistant to penicillin group of antibiotics, 66.6 percent (2/3) from sheep and 50 percent (1/2) poultry were resistant to both penicillin group and fluoroquinolone group (ciprofloxacin and enrofloxacin). Simultaneously, the samples subjected to isolation indicated the presence of *Staphylococcus pseudintermedius*, *Staphylococcus aureus*, *Streptococcus* spp, *E.coli Pseudomonas* spp. and *Klebsiella* spp. in more than 90 percent of the samples. The results of the nanoparticle based kit indicate that it can be effortlessly relied while choosing the antibiotics for the therapeutic management in field conditions.

Keywords: Antibiotics, sensitive, resistant, selection, canine

Introduction
Dogs are susceptible to various bacterial diseases like leptospirosis, staphylococcus, ehrlichiosis, brucellosis that could cause considerable mortality when not diagnosed or treated early but could be effectively managed if diagnosed and treated at the earliest (Patel *et al*., 2022) [9]. Among the enlisted bacterial infections like pyoderma, otitis, eye infections caused by are highly common in dogs. Antibiotics are the indispensable entity for the treatment of diseases caused by bacterial pathogens in animals and human community. Antibiotic susceptibility testing (AST) identifies effective antibiotic dosage and formulates a profile of empirical therapy for the proper management of an individual patient's health against deadly infections. The right choice of antibiotic is to treat these infections is a wise decision for a veterinarian to avoid unnecessary and indiscriminate usage of antibiotics which may lead to the emergence of antibiotic resistance (Lagier *et al*., 2015) [6]. The Clinical and Laboratory Standards Institute (CLSI) the most popular guidelines, which are based on provides pharmacokinetic–pharmacodynamic (PK-PD) properties and mechanisms of resistance (Kassim *et al*., 2016) [3]. Hence, there is a pressing need to prudently choose appropriate antibiotic to avert further complication including development of antibiotic resistance.
The conventional disc diffusion based antibiotic sensitivity test will take minimum two days (growth method) to maximum of four to five days to obtain by direct colony suspension method (Bayot and Bragg, 2022) [1] the results to choose the appropriate antibiotic (Sawatzky *et al*., 2015) [9]. Therefore, rapid diagnostic plays a pivotal role in the treatment of bacterial infection (Khan *et al*., 2019) [4]. The present study aims to rapidly identify the antibiotic suitable for the management of the infections caused by bacteria.

Materials and Methods
Samples Collection
A total of twenty-five swab samples in duplicate from dogs (n=15) with clinical conditions like pyoderma, otitis, nasal cavity and preoperative surgical sites, cattle (n=5) with mastitis

and abscess, goat with abscess, mastitis (n = 3), poultry (n = 2) with egg peritonitis and respiratory tract infections were collected from the clinical cases brought to Veterinary Clinical Complex, Namakkal during the year 2015.

Assessment of Antibiotic Sensitivity Pattern

The samples were collected using a sterile swab and rinsed immediately after collection in the sample suspension buffer provided in the kit. The required numbers of the kit components were brought to room temperature before use. The inoculum in the sample suspension buffer was injected into the enrichment media using a syringe provided in the kit and incubated for 4 hours at room temperature. Then one ml of the enrichment culture was injected into the each of the vial containing eight antibiotics viz., ciprofloxacin, ceftriaxone, ceftriaxone and tazobactum combination, ceftiofur, enrofloxacin, ampicillin and potassium clavulanate combination, ampicillin and dicloxacillin combination and finally amoxicillin and cloxacillin combination and control wells. The vials were incubated overnight at room temperature around 37 °C and the results were read as sensitive (colorless) and resistant (blue) based on the color change.

Isolation of bacteria

The second set of the swab samples collected from the animals were also inoculated in nutrient broth and incubated overnight at 37 °C in a bacteriological incubator to identify the type bacteria present in the sample. The inoculum from the nutrient broth were inoculated in mannitol salt agar and MacConkey agar and incubated overnight at 37 °C in a bacteriological incubator. The type of the bacteria was further confirmed by gram staining.

Results and Discussions

The clinical samples tested against eight antibiotics provided in the kit viz., ciprofloxacin, ceftriaxone, ceftriaxone and tazobactum combination, ceftiofur, enrofloxacin ampicillin and potassium clavulanate combination, ampicillin and dicloxacillin combination, amoxicillin and cloxacillin combination. Surprisingly, out of the 25 samples checked, 80 percent (12/15) from dog and 60 percent (3/5) from cattle were found to be resistant to penicillin group of antibiotics, 66.6 percent (2/3) from sheep and 50 percent (1/2) poultry were resistant to both penicillin group and fluoroquinolone group (ciprofloxacin and enrofloxacin). Simultaneously, the samples subjected to isolation indicated the presence of *Staphylococcus pseudintermedius*, in 90 percent of the ear or skin origin, few *Staphylococcus aureus* in the nasal swab samples of dogs and *Staphylococcus aureus along with either Streptococcus* spp, *Klebsiella* spp. *or Pseudomonas* spp. in more than 90 percent of the sample of bovine and sheep and goats causing mastitis and abscess formation.

The bacterial organisms inoculated in the mannitol salt agar produced gram positive cocci, yellowish colonies characteristics of *Staphylococcus aureus*, gram positive cocci colonies without any change in pH suggestive of *Staphylococcus pseudintermedius* and Gram negative rods producing rough colonies suggestive of *E.coli* and gram negative rods, mucoid colonies suggestive of *Klebsiella* spp. in MacConkey agar.

The results of the study indicate the *S. pseudintermedius* as the predominant pathogen associated with canine pyoderma, it is also the most common commensal species in dogs as reported by Lynch and Helbig 2021. Dogs are the most common animal species infected with *S. pseudintermedius*, with 84.7% of all *S. pseudintermedius* isolates originating from canine diseases including skin, ear and urinary tract infections (Ruscher *et al*., 2019) [8]. Zamankhan Malayeri *et al*. 2010 [12] reported that among total number of 92 isolated bacteria from dogs suffering with external otitis, 68 were *Staphylococcus pseudintermedius and remaining* were *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Escherichia coli*, *Pasteurella canis*. Further they also reported that all isolated gram-negative bacteria, were sensitive to amikacin and enrofloxacin, and had low resistance to ceftriaxone and gentamicin and were highly resistant to penicillin, eythromycin, and which are in harmony with findings of the present study. Van Duijkeren, *et al*., 2004 [11] describes dog's nares as the most frequently identified site of colonization of *Staphylococcus aureus* when cultures from several sites are performed which is similar to the findings of this study as these organisms could be isolated mainly from nasal swabs. (Table.1)

The bovine samples bacteria are similar to Kibebew 2017 [5] mentioning contagious pathogens such as *Staphylococcus aureus* and *Streptococcus agalactiae*, and less common species like *Mycoplama bovis* and *Corynebacterium*, live on the cow's udder and teat skin, colonizing and growing into the teat canal and are responsible for mastitis. A wide range of bacterial species were reported to cause environmental mastitis, namely, *Streptococcus* spp. (e.g. *Strep. uberis*), coliforms species (e.g. *E. coli*, *Klebsiella* spp., *Enterobacter* spp.), *Pseudomonas* spp., etc (Bogni *et al*., 2011) [2].

Table 1: Antibiotic sensitivity pattern tested with CanAntibiotic Quick Select kit and the bacteria isolated from the clinical samples

S. No.	Sample	Species	Antibiotics									Organism isolated
			Cip	Ceftr	Ceftr & Tazo	Ceftio	Enro	Amp & Pot. Clav	Amp & Clox	Amox & Clox	Control	
1.	Nasal Swab	Canine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. aureus*
2.	Nasal Swab	Canine	Sen	Sens	Res	Sen	Res	Res	Res	Res	No Change	*S. pseudintermedius*
3.	Pyoderma	Canine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. pseudintermedius*
4.	Pyoderma	Canine	Sen	Sen	Sen	Sen	Sen	Sen	Sen	Sen	No Change	*S. pseudintermedius*
5.	Aseptic Surgical wound	Canine	Sen	Sen	Sen	Sen	Sen	Res	Sen	Sen	No Change	*S. aureus & Klebsiella* spp.
6.	Nasal Swab	Canine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. pseudintermedius*
7.	Pyoderma	Canine	Sen	Sens	Res	Sen	Sen	Res	Res	Res	No Change	*S. pseudintermedius*
8.	Otitis	Canine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. pseudintermedius*
9.	Nasal Swab	Canine	Sen	Sens	Res	Sen	Res	Res	Res	Res	No Change	*S. aureus*
10.	Otitis	Canine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. pseudintermedius & Pseudomonas* spp.
11.	Otitis	Canine	Sen	Sens	Res	Sen	Res	Res	Res	Res	No Change	*S. pseudintermedius*

12.	Nasal Swab	Canine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. aureus*
13.	Skin swab	Canine	Sen	Sens	Res	Sen	Res	Res	Res	Res	No Change	*S. pseudintermedius*
14.	Nasal Swab	Canine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. pseudintermedius*
15.	Otitis	Canine	Sen	Sens	Res	Sen	Res	Res	Res	Res	No Change	*S. pseudintermedius*
16.	Mastitis Milk	Bovine	Sen	Sen	Sen	Sen	Sen	Sen	Sen	Sen	No Change	*Klebsiella* spp.
17.	Milk sample	Bovine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. aureus*
18.	Pus	Bovine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. aureus* *Streptococcus* spp. & Gram negative rods
19.	Milk sample	Bovine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. aureus & E.coli*
20.	Abscess	Bovine	Sen	Sen	Sen	Sen	Sen	Sen	Res	Sen	No Change	*S. aureus & Pseudomonas* spp.
21.	Mastitis Milk	Ovine	Res	Sen	Sen	Sen	Sen	Res	Sen	Sen	No Change	*S. aureus & Klebsiella* spp.
22.	Pus	Caprine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. aureus*
23.	Abscess	Caprine	Sen	Sen	Sen	Sen	Sen	Res	Res	Res	No Change	*S. aureus*
24.	Egg peritonitis	Poultry	Sen	Sen	Sen	Sen	Sen	Sen	Sen	Sen	No Change	*E.coli*
25.	Tracheal Swab	Poultry	Res	Sen	Sen	Sen	Res	Sen	Res	Sen	No Change	Very few colonies

Cip -Ciprofloxacin; Ceftr - Ceftriaxone; Ceftr & Tazo - Ceftriaxone & Tazobactum; Ceftio - Ceftiofur; Amp & Pot. Clav- Ampicillin & Pottassium Clavulanate; Amp& Clox -Ampicillin & Dicloxacillin; Amox& Clox -Amoxicillin& Cloxacillin; Enro- Enrofloxacin; Sen- Sensitive, Res- Resistant

Conclusion

The kit employs a novel concept of using a magnetic nanoparticle mediated entrapment of the bacteria and the test results are available within 11 h and read visually by colour change without the need for sophisticated instruments. The results of the nanoparticle based kit indicate that it can be effortlessly relied while choosing the antibiotics for the therapeutic management in field conditions wide variety of clinical samples apart from the canine origin for the veterinarians without sterile environment for the isolation of bacteria.

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