Microbiological Analysis and Antibiotic Sensitivity of Water for Wild Animals in Nandankanan Zoo, Odisha

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ABSTRACT

The incidence and prevalence of bacterial pathogens affecting zoo animals is increasing due to consumption of contaminated water containing the various persistent bacterial pathogens with increased antimicrobial resistance. The consumption of this antimicrobial resistance water causes transmission of several water borne bacterial diseases. Hence to save the lives of wild animals and to protect the ecological balance of our environment, a critical routine systemic analysis of supplied water with good monitoring practice and development of a database for routine screening of the water for captive animals is very much essential. So, the experiment was conducted to assess the microbial load in the form of CFU/ml and the identification of antibiotic resistant bacteria by antibiotic resistance test (ABST) in the supplied water from the enclosure pools at Nandankanan Zoo, Odisha. It was found that several bacterial isolates like *E. coli*, *Pseudomonas spp*, *Salmonella* and *Corynebacterium spp* are exclusively present in this contaminated water based on the cultural, morphological and biochemical characterization. Under antibiotic sensitivity test (ABST), tetracycline and sulfamethoxazole-trimethoprim was found resistant for *E.coli* in all the collected samples of wild animal species including birds and reptiles. Cephalothin and Sulfisoxazole were moderately resistance to *E. coli* in case of birds whereas Gentamycin and Neomycin were moderately resistance to the sample collected from Lions enclosure.

Keywords: Zoo animal, ecology, antibiotic, bacteria

Wildlife plays a major role in the ecological balance of our environment. The requirement of water is one of the basic needs of captive animals in zoos to perform their normal functions like fermentation and metabolism, proper flow of feed through the digestive tract, good nutrient absorption, normal blood volume and tissue requirements and health condition of young and adult animals (Grabow 1996). The contamination of water from agricultural runoff, effluents from septic systems or sewage discharges, infiltration of domestic or wild animal fecal matter now creates havoc. So, the presence of the indicator organisms such as *Escherichia coli*, Micrococcus, *Pseudomonas spp., Serratia spp., Flavobacterium spp., Chromobacterium spp., Acenobacter spp.* and *Alkaligenes spp.* makes water polluted and thus unsuitable for consumption which might cause different diseases in animals (Stephen and Joseph 2013). Though it is very difficult to treat an ailing animal in a safari, the antibiotics application in this case is an alternative. Antimicrobial agents are used therapeutically in animals and humans for control of bacterial infections. This practice is believed to enhance selection of resistant bacteria more than the therapeutic use of antimicrobial agents in response to clinical disease (Bogaard *et al.* 1999) and it may contribute to antimicrobial resistance in humans acquired through the human food chain (Barton 1998; Witte 1998). But due to antimicrobial resistance of several emerging pathogens, the wild animals in the zoo became novel reservoirs of zoonotic diseases of several bacterial species (Neu 1992; Cole *et al.* 2005; Sayah *et al.* 2005). As for example the geese are an important source of salmonellae, though they have been shown to shed large quantities of enterobacteria and campylobacters (Alderisio and Deluca 1999). One of the strategy to minimize this problem is water recycling scheme, a relatively simple

but highly effective innovation, which is maintained in different zoos of the world. Hence, the present study was carried out to determine the microbial load of water obtained at intervals from different enclosures of wild animals including birds. Simultaneously, considering *E. coli* as the indicator organism, antibiotic sensitivity test (ABST) was conducted for routine screening of the water for captive animals in the Nandankanan zoo, Odisha.

MATERIALS AND METHODS

A total of 36 water samples from different enclosures consisting of 8 tigers, 14 lions, 5 birds and 9 reptiles were collected from the main water source, storage source and captive enclosures up to 120 days at various intervals. The bacterial load and the presence of indicator organisms in the collected water sample were determined as per the CFU/ml and the detection of CFUs in a water sample was carried out by standard plate count method of Prescott and Harley (2002). The detection of total coliforms in a water sample using the multiple tube fermentation method was conducted as per the standard method prescribed by Collee *et al*. (1996). A total coliform count was performed by the membrane filtration technique as per the standard protocol (Bordner and Winter 1978). Results were expressed as total coliforms per 100 ml of water (American Public Health Association, 1971). The number of fecal coliform counts per 100 ml of water sample was calculated as per Brenner *et al*. (1996). The water sample after membrane filtration (0.45 μ) were enumerated *pseudomonas spp.* according to the protocol of Sutter, (1968). Antibiogram of *E. coli* isolated from various sources of water samples such as faeces, environment and septage was carried out as per the standard protocol of Klein and Bulte (2003). The statistical analysis of the data was done according to Snedecor and Cochran (1994). The data analyzed for analysis of variance (ANOVA) and DMR test (Duncan, 1955) was used to test the difference in treatment means.

RESULTS AND DISCUSSION

Coliform profile of water

All the collected samples from various enclosures were subjected to microbial profiling study. The mean coilform count in 8 tiger enclosures was found to be 230 ± 40 CFU/ml at the end of the week of water supply. 14 samples from lion enclosures showed the mean coliform count of 210 ± 40 CFU/ ml and the samples from reptiles $> 290 \pm 30$ CFU/ ml, whereas coliform count of wild bird enclosure water analysis was found to be 180 ± 20 CFU/ ml. The water sample of the main source of water supply tested for coliform count both at the start and at the end of the week showed no change varying from 7 ± 2 CFU/ml. The high population of coliform bacteria confirms the pollution of water resources at various sources (Grabow, 1996). Results obtained in this study indicated that for routine water quality monitoring, EMB and MLA agar remain the methods of choice for enumerating fecal coliforms in water. EMB agar would be the most suitable approach for the specific enumeration of *E. coli*. The selectivity of the media used with the various methods appears to be more ideal (Dionisio and Borrego 1995) and it is in agreement with our present study. The presence of *E. coli* in water also indicates the risk of infection to users with respect to public health.

Various bacterial species profile of water

The water samples invariably showed the presence of several species of bacteria based on the cultural and biochemical characterization (Table 1). The presence of *E. coli* was demonstrated by the formation of acid and gas from lactose or mannitol at 44ºC and indole from tryptophan at 44ºC and oxidase negative. The presence of *E. coli* in water also indicates the risk of infection to users. *P. aeruginosa* and other *Pseudomonas* spp. were confirmed by production of pigment and casein hydrolysis upon milk

Table 1: Microbial population in different enclosures

Species	Total coliform *spp.*	*Pseudomonas spp.*	*Salmonella spp.*	*Corynebacterium spp.*	Fecal coliform
Tiger (N=8)	230 ±40 CFU/ml.	55%	100%	100%	90%
Lion (N=14)	210 ±40 CFU/ml.	60%	100%	100%	90%
Bird (N=5)	180 ±20 CFU/ml.	80%	100%	100%	50%
Reptile (N=9)	290 ±30 CFU/ml.	80%	100%	100%	100%

agar with cetrimide after 24 hrs at 44°C. *Salmonella spp.* was culturally characterized by black centered colonies against *Corynebacterium* spp. on differential Hektoen Enteric Agar (HEA) media. The microbial population present in various wildlife enclosures at 90 and 120 days of water supply were detected and found to be 100% of *Salmonella spp* and *Corynebacterium spp.* The faecal coliform and *Pseudomonas* spp. were comparatively lesser which were 82% and 70% across all the species. The prevalence of food borne pathogens *E. coli* is the major water borne outbreak and has been documented from a number of countries (Bartlett 1996; Ogden *et al.* 2001). So based on this study the various food borne pathogens identification was done as per their standard methods described above. It was found that, *E. coli* was one of the major pathogens detected in the water samples tested.

Table 2: Patterns of antimicrobial agent resistance in *E. coli* isolated from various sources (feces, environment and septage); R= Resistant, MR= Moderately Resistant, S=Sensitive or Susceptible

Antimicrobial agent	Tiger (N=8)	Lion (N=14)	Birds (N=5)	Reptiles (N=9)
Neomycin	R	MR	S	S
Gentamicin	MR	MR	S	S
Streptomycin	R	R	S	S
Chloramphenicol	MR	R	S	R
Ofloxacin	R	S	S	R
Sulfamethoxazole-Trimethoprim	R	R	R	R
Tetracycline	R	R	R	R
Ampicillin	S	R	S	R
Nalidixic acid	R	R	S	R
Nitrofurantoin	R	R	S	R
Cephalothin	R	R	MR	R
Sulfisoxazole	R	R	MR	R

Pattern of antibiotic resistance

The tetracycline and sulfamethoxazole-trimethoprim were the most resistance in all the samples followed by cephalothin, sulfisoxazole and streptomycin. Out of the 12 antimicrobial agents tested, 10 showed the patterns of antimicrobial resistance for *E. coli* within tiger enclosure (Table 2). Comparing the resistance between tetracycline and trimethoprim-sulfamethoxazole in *E. coli* isolates, it

was found that resistance to tetracycline was present in samples from all species, while resistance to trimethoprim-sulfamethoxazole was present in all types of samples from wild birds. Disk diffusion zone was also examined for determining different pattern of antibiotic sensitivity among types of samples collected. Significant differences were seen in the sizes of diffusion zone for all agents except tetracycline and sulfisoxazole.

Overall, the largest diffusion zone (indicating greater susceptibility) was found with *E. coli* isolates. The exceptions were the diffusion zones for tetracycline, ampicillin and sulfisoxazole; for these agents the water isolates had the smallest diffusion zones. Bird isolates and water isolates had the smallest diffusion zones for all agents except neomycin, gentamicin, nitrofurantoin and cephalothin. This antimicrobial resistance is grown up in the last few decades resulting in increased morbidity, mortality and health care cost (Cohen 1992). Injudicious use of antibiotics is the prime culprit for the emergence of antimicrobial resistance pathogens in both human and veterinary health background (Smith *et al.* 2005; Verma *et al.* 2007).

Therefore, there is a need for continuous surveillance of antimicrobial resistance trends particularly among the resident bacterial pathogens in the gastrointestinal tract of wild animals. The implementation of antibiotic use strategies by ABST will decrease the risk and the clinical threat posed by antimicrobial resistance at all levels. It was also revealed that from all the enclosures of water sample, the presence of *E. coli as* indicator organisms, was the cause of several bacterial diseases and became the reservoir of infection under the antibiotic pressure. Hence, the administration of judicious and recommended dose of antibiotics by following this ABST result by zoo veterinarian is highly essential. The waste disposal system along with water recycling issues is strictly to be followed near the premises of zoo to maintain the proper hygiene and sanitation of wild animals. Additional research is needed to address this question, including expanding the collection of samples to other potential sources of resistant bacteria and comparing the genetic characteristics of bacteria surviving in the environment.

CONCLUSION

Several bacterial isolates like *E. coli*, *Pseudomonas spp*,

Salmonella and *Corynebacterium spp* are exclusively present in this contaminated water based on the cultural, morphological and biochemical characterization. Under antibiotic sensitivity test (ABST), tetracycline and sulfamethoxazole-trimethoprim resistant *E.coli* was found in all the collected samples of wild animal species.

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