Provisional PDF

Published 25 may 2010

Research, Volume 5, Issue 12, 2010

Microbial evaluation and public health implications of urine as alternative therapy in clinical pediatric cases: health implication of urine therapy

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Abstract

BACKGROUND: Cultural means of pediatric treatment during ill health is a mainstay in Africa, and though urine has been known to contain enteric pathogens, urine therapy is still culturally applicable in some health conditions and also advocated as alternative therapy. The study therefore, is to evaluate the microbial contents and safety of urine. **METHODS**: Urinary bacteria from cows and healthy children aged 5-11 years were identified by conventional phenotypic methods and antimicrobial susceptibility testing was performed using modified agar disc and well-diffusion methods. **RESULTS**: A total of 116 bacterial isolates (n = 77 children; n = 39 cows) were identified as Bacillus (10.4%; 5.1%)), Staphylococcus (2.6%; 2.6%), Citrobacter (3.9%; 12.8%), Escherichia coli (36.4%; 23.1%), Klebsiella (7.8%; 12.8%), Proteus (18.2%; 23.1%), Pseudomonas (9.1%; 2.6%), Salmonella (3.9%; 5.1%) and Shigella (7.8%; 12.8%) spp. Antibiotic resistance rates of the Gram-positive bacteria were high (50.0-100%), except in Bacillus strains against chloramphenicol, gentamicin and tetracycline (14.3%), while higher resistance rates were recorded among the Gram-negative bacteria except in Citrobacter (0.0%) and Proteus (8.5%) spp. against gentamicin and tetracycline respectively. The Gram-negative bacteria from ito malu (cow urine) were more resistant bacteria except in Citrobacter (20.0%) and Shigella spp. (0.0%) against tetracycline and Proteus spp. (11.1%), (22.2%) against amoxicillin and tetracycline respectively. Multiple antibiotic resistance (MAR) rates recorded in children urinal bacterial species were 37.5-100% (Gram-positive) and 12.5-100% (Gram-negative), while MAR among the cow urinal bacteria was 12.5-75.0% (Gram-positive) and 25.0-100% (Gram-negative). Similar higher resistance rates were also recorded among the Gram-negative bacterial species from urine specimens against the paediatric antibiotic suspensions. **CONCLUSION**: The study reported presence of multiple antibiotic-resistant indicator bacteria in human urine and ito malu used as alternative remedy in pediatric health conditions like febrile convulsion

Background

There are various family behavioral practices in medicine and health but some of the traditional healing practices in Africa include herbal therapies [1] and other forms of alternative therapies which are non-clinical. Traditional home remedies are still commonly used in childhood ill-health conditions like convulsions in Nigeria, and the administered remedy depends on the practices of the community [2]. Based on certain traditional points of view, consumption of *ito malu* (cow urine) and human urine is a common form of traditional remedy for convulsion and related health conditions in children in some cultural settings in Nigeria. Similarly, current propaganda on urine therapy has also started to rise. The practice of using urine in traditional medicine and healthcare in parts of Nigeria are scantily reported but it is known that some parents use human and cow urine as a form of folk medicine in paediatric ill health, most especially in cases of febrile convulsion; the most common type of convulsion in childhood, which is usually frightening to parents [3] and which according to the National Institute of Health Consensus Statement [4] is defined as an event in infancy or childhood, usually occurring between 3 months and 5 years of age, and associated with fever but without evidence of intracranial infection or defined cause for the seizure.

Lack of proper delivery of health care services in the country has strongly supported the increase in any form of alternative therapy in the country, including the advocacy for urine therapy. Kafaru [5] stated that many of the Nigerian older people used human urine in serious cases of ill-health such as wound sores, diabetes and even when poison was suspected; while cow urine is mixed with herbal preparation for treating convulsion in babies. Shodipe [6] reported that in traditional treatment of sickle cell anemia, grinded leaves of tobacco (*Nicotiana tabacum*) are soaked in six pints of cow urine and six bottles of aromatic schnapps gin, then 1 desert spoon of rock salts is added to the mixture in a big bottle corked and kept for 2 days. Adodo [7] also reported that urine therapy can cure cancer. Narrating a personal experience in using auto-urine for therapy, Kafaru [5] reported that in 1980, when she was bleeding during a four-month pregnancy, she drank only urine and water to stop the bleeding after six hours. She was of the opinion that urine therapy is the most curative, easy-to-use and most cost effective.

Previous studies however, had reported that urine usually contained many enteric bacteria and that most urinary tract infections (UTIs) are caused by enteric bacteria [8-14]. Possible bacterial pathogens that are found in urine include enterococci, such as *Enterococcus faecalis, Bacillus, Staphylococcus saprophyticus*, haemolytic streptococci, such as *Streptococcus agalactiae, Citrobacter spp*., *E. coli, Enterobacter aerogenes, Enterobacter cloacae*, coagulase-negative staphylococci, Proteus spp., such as *Proteus mirabilis, Pseudomonas aeruginosa*, Klebsiella spp., such as *Klebsiella pneumoniae, Klebsiella oxytoca, Acinetobacter* species and diptheroids, while yeasts like *Candida* spp. may also be found in the female urethra, caused by contamination of the urine with the skin. Commensals including staphylococci and Mycobacterium smegmatis may also occur as specimen is being collected [11, 15-16]. It is quite true that most African countries have traditional means of curing clinical conditions; however, it is very fundamental and necessary that adequate measures are taken to address cultural health situations that are known to pose great risks, especially to children. It is thereby necessary that treatment of infectious or non-infectious diseases, especially of public health importance using traditional remedies should be supported by scientific evidence. This present study therefore, tries to provide information from the microbiological point of view, on the public health significance of urine of children and cows as alternative therapy in pediatric cases, and to serve as a forum for the exchange of ideas and clarification on this speculated traditional means of curing pediatric related ill health conditions like convulsion.

Methods

Samples' collection and analyses

Microbiological cultures were performed on aseptically collected 100 early morning midstream urine specimens of healthy children (53 female, 47 male), aged 5-11 years in two state capitals of Nigeria, Abeokuta (Ogun state) and Ibadan (Oyo state). The subjects were not on antibiotic therapy within 3 months prior to the period of specimens' collection. The collection process was without any inclusion or exclusion criteria, such as acute voiding symptoms, significant bacteriuria with growth of at least 10 colonyforming units/ml urine, leukocyturia > 50/ µl, underlying renal diseases, anatomic abnormalities of the urinary tract, like in the study of Pape et al. [11]. Consenting parents were trained on how to collect freshly-voided midstream urine specimens of their children. The cow urine specimens were personally collected from Bodija and Moniya abattoirs, after obtaining the consents of the personnel in charge. All the urine specimens were cultured within 4 hours on same days of collection.

Isolation and characterization of bacterial species from the urine specimen

Urine culture were determined on plate count agar (PCA; LAB M), eosin methylene blue agar (EMB), MacConkey (MCC; LAB M) agar and cystein lactose electrolyte deficient (CLED; LAB M) agar at 350 degree Celsius for 24-48 hours. Different colonies on culture plates having ≥1.0 x 104 cfu ml-1 were randomly selected and colonies sub cultured on CLED and MacConkey agars. Pure bacterial isolates were identified to species level by the standard taxonomic protocol of the laboratory, based on the phenotypic profiles, including their cultural and microscopic morphologies as well as basic biochemical procedures [17].

Antibiotic susceptibility determination (discs)

The antimicrobial susceptibility testing on the bacterial isolates was performed using the disc diffusion method. The antibiotics included in the determination of antibiotic resistance in this study were the range of antibiotic classes commonly imported into the country (Amoxicillin (25µg), Augmentin (30µg), Cloxacillin (5µg), Erythromycin (5µg), Chloramphenicol (30µg), Cotrimoxazole (25µg), Gentamicin (10µg) and Tetracycline (10µg), while for the Gram-negative bacterial strains were- Penicillin (10µg), Clindamycin (20µg), Gentamicin (10µg), Fusidic acid (10µg), Erythromycin (5 µg), Trimethoprim (25µg), Sulphamethaxazole (25µg) and Tetracycline (10µg)) and routinely used in the treatment of UTIs. Zones of inhibition were measured and the diameter recorded in millimeter. Zones less than 10.0 mm in diameter or absence of inhibition zones were recorded as resistant (negative) [18-19]. The antibiotic discs were obtained from ABTEK Biologicals Ltd. (Liverpool, UK).

Antibiotic susceptibility determination (pediatric suspensions)

The twenty one pediatric antibiotic suspensions, ampicillin (Emicillin; 125mg/5ml), amoxicillin/Clavulanic acid (Fleming; 228.5 mg/5ml), metronidazole (Ioxagyl; 200 mg/5ml), cotrimoxazole (Septrin; 240 mg/5ml), cefuroxime axetil (Zinnat; 125 mg/5ml), amoxicillin (Amaxin; 125 mg/5ml), Fluiloxacillin (Floxapen; 125 mg/5ml), azithromycin (Zithromax; 200 mg/5ml), ampicillin / cloxacillin (Jawaclox; 250 mg/5ml), Erythromycin (Erythrokid; 250 mg/5ml), Cefalerin (Cefamor; 125 mg/5ml), Erythromycin (Etocin; 200 mg/5ml), Erythromycin (Throtal; 125 mg/5ml), Cotrimoxazole B.P. (Loxaprim; 240 mg/5ml), Cotrimoxazole

(Rancotrim; 240 mg/5ml), Cotrimoxazole (200 mg/5ml), Chloramphenicol (Clofencol; 125 mg/ 5ml), Cefaclor (Vercef; 125 mg/5ml), ampicillin + cloxacillin (Emzoclox; 250 mg/5ml), Sulfamethoxazole + Trimethoprim (Bactrim; 240 mg/5ml) and Cefadroxil (Odoxil DS; 250 mg/5ml) were assayed for in this study using the modified [20] agar well-diffusion method of Tagg et al. [21]. Sterile Mueller-Hinton agar was poured into sterile Petri dishes and allowed to set. Wells about 6.0 mm were bored into the agar followed by surface sterilization of the agar plates by flaming. The entire surface of each of the sterile Mueller-Hinton agar plates was then streaked with each bacterial isolate using sterile swab sticks. The plates were left for about 10 minutes before aseptically dispensing the pediatric antibiotic suspensions (antibiotic powder dissolved in recommended volume of sterile distilled water) into the agar wells. The plates were then incubated in upright position at 350C for 24 hours. Zones of inhibition were measured and the diameter recorded in millimeter. Although, the interpretations of the inhibitory zones of antimicrobial agents like antibiotics vary, however, just like some earlier workers, zones of inhibition less than 10.0 mm in diameter or absence of inhibition zones were recorded as resistant (negative) [18, 19].

Results

The pH of the children urine specimens were between 5.5 and 14.0 (Abeokuta) and 6.0 and 13.0 (Ibadan) but more of the urine specimens had pH of 6.5 (14.0%), 7.0 (21.0%) and 6.0 (27.0%). Other pH values were 7.5 (7.0%), 8.0 (9.0%), 8.5 (5.0%), 9.0 (5.0%), 9.5 (3.0%), 12 and 14 (1.0%). The pH of the cow urine samples were 7.5 (12.1%), 8.0 (54.5%), 8.5 (24.2%) and 9.0 (9.1%). Total colony counts of the urine specimens were between 1.0 x 10^2 and 1.2 x 10^8 cfu ml^{-1} on CLED; 1.5 x 10^2 and 8 x 10^7 cfu ml^{-1} on MCC; 2.2 x 10^3 and 1.2 x 10^5 cfu ml^{-1} on EMB agar plates. The counts on PCA were too numerous to count on most of the culture plates at dilutions between 10^{-2} and 10^{-4} while at dilutions of 10^{-5} to 10^{-6}, countable colonies of 1.4 x10^6 - 1.2 x 10^7 cfu ml^{-1} were obtained.

Apart from fungal colonies that were obtained from the urine specimens, 77 bacterial strains were randomly isolated from 100 children urine specimens, while 39 bacterial strains were isolated from 33 urine specimens of cows. The identified Gram-positive bacterial isolates obtained from the children urine specimens were *Bacillus* 8 (10.4%) and *Staphylococcus* 2 (2.6%) species, while Gram-negative bacterial species were *Citrobacter* 3 (3.9%), *Escherichia coli* 28 (36.4%), *Klebsiella* 6 (7.8%), *Proteus* 14 (18.2%), *Pseudomonas* 7 (9.1%), *Salmonella* 3 (3.9%) and *Shigella* 6 (7.8%) (Table 1). Only *Salmonella*, *Shigella* and *Staphylococcus* species were not obtained from Abeokuta urine specimens. As shown in Table 2, the bacterial species from the urine specimens of cows were *Bacillus* 2 (5.1%), *Staphylococcus* 1 (2.6%), *Citrobacter* 5 (12.8%), *Escherichia coli* 9 (23.1%), *Klebsiella* 5 (12.8%), *Proteus* 9 (23.1%), *Pseudomonas* 1 (2.6%), *Salmonella* 2 (5.1%) and *Shigella* 5 (12.8%).

In this study, the antibiotic resistance patterns of the bacterial isolates from the urine specimens of children (antibiotic discs) indicated that the antibiotic resistance of the Gram-positive bacterial species (*Bacillus* and *Staphylococcus*) were high (50.0-100%), except in *Bacillus* strains against chloramphenicol, gentamicin and tetracycline (14.3%). Higher resistance rates were also recorded among the Gram-negative bacteria against the test antibiotics except in *Citrobacter* (0.0%) and *Proteus* (21.4%) towards gentamicin. Multiple antibiotic resistance recorded among the Gram-positive bacterial species were 37.5-87.5%, while 12.5-100% MAR were recorded among the Gram-negative bacterial species from the urine specimens of children. At least, thirteen of the bacterial strains had total resistance (100%), while only maximum of five strains had total (100%) susceptibility towards all the test antibiotics (Table 1).

The *Bacillus* strains from the urine specimens of cows were more resistant to the test antibiotics (50.0-231 100%), except in chloramphenicol, in which no resistance was recorded. Similarly, the resistance patterns of the Gram-negative bacterial species from urine of cows were more resistant towards the test antibiotics (40.0-100%), except in *Citrobacter*, *Klebsiella* and *Proteus* against gentamicin (20.0%; 20.0%, 11.1%) and *Citrobacter* and *Proteus* against tetracycline (20.0%; 22.2%) respectively. No resistance was recorded among the *Shigella* strains against tetracycline. The MAR recorded among the bacterial species from the urine specimens of cows in this study was between 12.5 and 100%. Six of the bacterial strains from urine specimens of cows had total (100%) susceptibility, while only two strains had total (100%) resistance towards all the test antibiotics (Table 2).

As shown in Table 3, all the *Bacillus* and *Staphylococcus* strains from children urine were moderately or highly resistant to the pediatric antibiotic suspensions except in ampicillin/ cloxacillin (Emzoclox) and flucloxacillin (Floxapen) (12.5%); azithromycin (Zithromax) cotrimoxazole (Ranotrim), erythromycin (Erythrokid) and sulfamethoxazole/trimethoprim (Bactrim) (25.0%) to which the *Bacillus* strains were more susceptible. Total susceptibility was recorded in *Bacillus* strains towards ampicillin/cloxacillin (Jawaclox), in *Staphylococcus* strains towards amoxicillin/ clavulanic acid (Fleming), ampicillin/cloxacillin (Jawaclox) and azithromycin (Zithromax).

Relatively higher resistance rates were recorded by the bacterial species from urine of cows towards the test paediatric antibiotics although total (0.0%) susceptibility were exhibited towards few of the bacterial strains by some paediatric antibiotics [Amoxicillin/clavunlanic acid (Fleming), Ampicillin (Emcillin), ampicillin\cloxacillin (Emzoclox), ampicillin\cloxacillin (Jawaclox), Azithromycin (Zithromax), Cefaclor (Vercef), Cefalexin (Cefamor), Cefuroxine (Amaxin), Chloramphenicol (Clofencol), cotrimoxazole (Septrin), Cotrimoxazole B.P (Loxaprim), cotrimoxazole (Rancotrim), erythromycin (Erythrokid), erythromycin (Etocin), Metronidazole (Loxagyl), sulfamethoxazole/trimethoprim (Bactrim) (Table 4).

Multiple antibiotic resistance patterns were also exhibited by the Gram positive bacterial strains from the urine of children (*Bacillus*; 28.6 – 61.9%) and (*Staphylococcus*; 38.1 – 81.0%) against the pediatric antibiotic suspensions (Table 3), while as high as 81.0% MAR was also exhibited by the bacterial species from cows towards the pediatric antibiotic suspensions (Table 4).

Discussion

Febrile seizures, which carry a good prognosis around the world are associated with a relatively high mortality and morbidity in Africa and other courtiers of the world [22,23]. Convulsion among children between six months and five years is a major contributor to childhood mortality in less-developed societies, especially in sub-Saharan Africa [24]. In Nigeria, this has been attributed to the administration of some indigenous concoctions before the children are brought to hospital [25-27]. The traditional concoctions used in the treatment of convulsion vary with cultural practices [26], and based on the parental fears in cases of seizures, as well as accumulating epidemiological evidence indicating that febrile seizures are the most common recognized antecedent for epilepsy in childhood [28-30], various home remedies including human and cow urine, kerosene, fuel and crude oil are used in cases of infantile cases of febrile convulsion [2,25,26].

In this study, apart from the viable and culturable Gram-positive bacterial species (Bacillus and Staphylococcus), the most prevalent Gram-negative bacterial species obtained from the children urine were *E. coli* (36.4%) and *Proteus* (18.2%), although other Gram-negative bacterial uropathogens, *Citrobacter, Salmonella, Klebsiella, Pseudomonas* and *Shigella* were also isolated, in agreement with previous studies on the aetiology of pediatric uropathogens [9,13,14,16,31-33]. *Citrobacter, Klebsiella, Proteus, E. coli* and *Shigella* were also the most recovered bacterial species from urine of cows in this study. These groups of pathogens are also similar to those obtained from some earlier studies [12,34,35]. Recovery of these group of indicator bacteria from urine that are consumed for the cure of clinical conditions in children is therefore, of great concern, especially because the isolated bacterial pathogens have been implicated in infantile and children gastroenteritis in some earlier studies [36-42]; and it is a well known fact that diarrhoeal diseases are a principal cause of childhood morbidity and mortality in the developing countries like Nigeria, being responsible for death of more than 4 million children each year [43-45].

It has also been well reported that antibiotic resistance demonstrates considerable geographic variability [31,45,47], while studies on pediatric uropathogens in most countries also indicated a rise in resistance to common antibiotics and continuing evolution of resistance to antimicrobial agents, as well as large inter-regional variability [9,13,31-33,48-50]. Moderate to high resistance (26-63%) to ampicillin, amoxicillin-clavulanic acid, cephalothin, cefuroxime and trimethoprim-sulfamethoxazole (SMZ/TMP) was noted among some of the bacteria in this study, which is similar to some earlier studies [13,33,51,52]. In the study of Pape et al. [11] as well, resistance rates to cotrimoxazole and 1st generation cefalosporines increased by about 20% compared with the previous analyses undertaken between 1990 and 1995, and therefore, concluded that the policies for treatment of UTI in children should be re-evaluated every 5 years according to local resistance rates. As an example, SMZ/TMP is a popular antibiotic in the treatment of pediatric UTIs and other clinical cases but the Infectious Disease Society of America had stated that with an SMZ/TMP resistance of 10–20% in adults, alternative first-line antimicrobial agents should be used [53]. This is based on adult uropathogen data but it is difficult to determine whether this same cut-off can be used in paediatric populations. Higher resistance (17.9-100%) to SMZ/TMP in this study therefore, raises some concerns.

Most of the bacteria isolated from urine of cows in this study also exhibited high rates of MAR towards 4 or more number of antibiotics. Globally, an estimated 50% of all antimicrobials serve veterinary purposes and literature of the last few years provides ample evidence that antibiotic resistance traits have entered the microflora of farm animals and the food produced from them [54]. Antimicrobial resistance has also emerged in zoonotic enteropathogens, commensal bacteria and bacterial pathogens of animals, although the prevalence of resistance varies [46,47,54]. In this present study, the recorded resistance rates as high as 87.5-100% observed among the bacterial species from urine of children, in spite of the fact that the bacterial species were non-UTI confirmed pathogens signifies that the introduction of strains that are drug resistant into a community plays a greater role in changing the prevalence of drug-resistant UTI [55]. In the five-year retrospective study by Ladhani and Gransden [55], bacterial isolates from children with underlying renal problems were generally more resistant to commonly used antibiotics in comparism with the children in the community, therefore, if such high antibiotic resistance (87.5-100%) as reported in this study were recorded among non-UTI confirmed bacterial strains, it then means that usage of urine of children and cows as alternative therapy in pediatric ill-health conditions like convulsion is quite hazardous.

Children are the most vulnerable members of any society since their immunity is not fully developed [45] but though the premium placed on children in African societies is so high, yet, even in the 21st century, the childhood mortality rates in the majority of African countries remain disturbingly high, with some 12 million children under–5 dying every year [56]. In 2006, there were 41 countries in which at least 10% of children under five died and all but three of the countries were in Africa. Ten of the 41 countries had higher rates of child mortality than 1990 and four were exactly the same. Among the worst 20, Nigeria ranked 12th with 181 deaths per 1000 [57]. Though the expected benefits of medical intervention should outweigh the possible harm, parents and guardians occasionally adopt some interventions which are futile, harmful and with no apparent curative or pathophysiologic rationale [25,58,59]. In Nigeria, as in most other developing countries, children are subjected to unorthodox treatment as first aid therapy in emergency conditions at home. In a study by Iyun and Tomson [60] in Nigeria, the reported dominating practice of mothers in cases of acute respiratory infections of children was either the use of irritants to get rid of the cause of the disease (‘coldness’) through vomiting, by forcing the child to swallow bitter remedies such as cow urine, or to use a remedy with warming and soothing properties.

Conclusion

Urine therapy is being advocated worldwide as an alternative therapy in many clinical cases. This study however, confirms that there could be introduction of multiple antibiotic resistant bacterial pathogens through consumption of cow and human urine in pediatric cases, more especially, since urine has not been reported to be of any medical benefit in cases of convulsion or other pediatric health condition. Although the magnitude of antibiotic resistance vary among regions but the rates are alarmingly higher in the developing countries. This study has been able to highlight the public health implications of urine as alternative therapy in paediatric convulsion in a developing country like Nigeria, which is of great concern. Only the non-fastidious, viable and culturable bacterial species were isolated in this study, indicating that more pathogenic strains can be isolated with more sophisticated culture media and kits. It is strongly suggested that alternative therapies should be non-hazardous, and therefore, inappropriate administration of remedies, such as urine therapy in pediatric health conditions should be discouraged, considering the fact that no documented scientific / clinical evidence of the beneficial effect of urine therapy in clinical had been reported, while multiple antibiotic resistant bacterial species had also been recovered from such urine.

Competing interests

The authors declare no competing interest in the study.

Authors' contribution

V.A. Ajayi collected urine specimens from human subjects at Abeokuta locations and carried out some preliminary studies on the specimens. **A.O. Fawole** collected urine specimens from human subjects and cows at Ibadan locations, carried out some advanced studies on the specimens and wrote the draft of the results. **A.A.O. Ogunshe** supervised the collection of urine specimens at Abeokuta and Ibadan locations, carried out advanced studies on the specimens, corrected and edited the final write up of the manuscript.

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Table 1: Antibiotic resistance patterns of isolated bacterial flora from urine samples of children using antibiotic discs

	% Antibiotic resistance (µg) / disc								
Isolates	**AUG**	**AMX**	**ERY**	**TET**	**CXC**	**GEN**	**COT**	**CHL**	**MAR**
Bacillus spp. [8]	87.5	100	57.1	14.3	100	14.3	57.1	14.3	(37.5 – 87.5)
Staphylococcus sp. [2]	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	(100.0)1*1±
	PEN	**CLN**	**GEN**	**FUS**	**ERY**	**TRM**	**SMX**	**TET**	
Citrobacter spp. [3]	100	100	0.0	100	100	33.3	33.3	33.3	(50.0 – 75.0)
E. coli [28]	85.7	60.7	28.6	60.7	67.9	60.7	67.9	46.4	(12.5–100)2*5±
Klebsiella spp. [6]	100	66.7	33.3	50.0	66.7	100	83.3	33.3	(37.5 – 87.5)
Proteus spp. [14]	71.4	57.1	21.4	50.0	50.0	64.3	64.3	8.5	(12.5–100)2*3±
Pseudomonas spp. [7]	100	87.5	28.6	71.4	71.4	71.4	71.4	42.9	(37.5 -100) 2±
Salmonella spp. [3]	100	66.7	66.7	66.7	66.7	100	100	33.3	(37.5 – 100.0)1±
Shigella spp. [6]	100	66.7	16.6	33.3	83.3	66.7	33.3	33.3	(25.0 -100)1±

AUG: Augmentin, AMX: Amoxycillin, ERY: Erythromycin, TET: Tetracycline, CXC: Cloxacillin, GEN: Gentamicin, COT: Cotrimoxazole, CHL: Chloramphenicol, PEN: Penicillin, CLN: Clindamycin, GEN: Gentamicin, FUS: Fusidic acid, ERY: Erythromycin, TRM: Trimethoprim, SMX: Sulphamethaxazole, *: total susceptibility, ± : total resistance

Table 2: Antibiotic resistance of isolated bacterial flora from urine samples of cows using antibiotic discs

	% Antibiotic resistance (µg) / disc								
Isolates	**AUG**	**AMX**	**ERY**	**TET**	**CXC**	**GEN**	**COT**	**CHL**	**MAR**
Bacillus spp. [2]	100	100	100	100	100	50.0	50.0	0.0	(75.0)
Staphylococcus spp. [1]	0.0	0.0	0.0	100	0.0	0.0	0.0	0.0	(12.5)
	PEN	**CLN**	**GEN**	**FUS**	**ERY**	**TRM**	**SMX**	**TET**	
Citrobacter spp. [5]	80.0	80.0	20.0	80.0	80.0	60.0	40.0	20.0	(50.0–100.0) 1*1±
E. coli [9]	100	88.9	33.3	77.8	100	66.7	33.3	55.6	(50.0–100.0) 2*
Klebsiella spp. [5]	80.0	100	20.0	100	100	100	80.0	60.0	(62.5–100.0) 1*
Proteus spp. [9]	88.9	55.6	11.1	44.4	55.6	100	88.9	22.2	(37.5–100.0) 1*
Pseudomonas spp. [1]	100	100	100	100	100	100	100	100	(100.0) 1*
Salmonella spp. [2]	100	100	100	50.0	100	50.0	100	50.0	(62.5–100.0) 1±
Shigella spp. [5]	80.0	80.0	40.0	40.0	60.0	80.0	60.0	0.0	(25.0- 87.5)

AUG: Augmentin, AMX: Amoxycillin, ERY: Erythromycin, TET: Tetracycline, CXC: Cloxacillin, GEN: Gentamicin, COT: Cotrimoxazole, CHL: Chloramphenicol, PEN: Penicillin, CLN: Clindamycin, FUS: Fusidic acid, ERY: Erythromycin, TRM: Trimethoprim, SMX: Sulphamethaxazole, *: total susceptibility, ±: total resistance

Table 3: Antibiotic resistance pattern of isolated bacterial flora from urine samples of children using pediatric oral suspensions (mg/ml)

	% Antibiotic resistance							
Antibiotic suspensions mg/ml)	***B. ce r* [8]**	***Staph* [2]**	***Citro* [3]**	***E. coli* [27]**	***Kleb* [6]**	***Prot* [14]**	***Salm* [3]**	***Shig* [6]**
Amoxicillin (Amaxin)	50.0	50.0	100.0	35.7	50.0	35.7	33.3	50.0
Amoxicillin / clavunlanic acid (Fleming)	50.0	0.0	100.0	28.6	66.7	35.7	33.3	33.3
Ampicillin (Emcillin)	75.0	50.0	33.3	28.6	50.0	14.3	66.7	33.3
Ampicillin/ cloxacillin (Emzoclox)	12.5	50.0	66.7	14.3	33.3	21.4	0.0	16.7
Ampicillin / cloxacillin (Jawaclox)	0.0	0.0	100	28.6	50.0	28.6	33.3	33.3
Azithromycin (Zithromax)	25.0	0.0	66.7	25.0	50.0	28.6	33.3	33.3
Cefaclor (Vercef)	50.0	50.0	66.7	53.6	50.0	35.7	33.3	16.7
Cefadroxil (Odoxil DS)	75.0	50.0	66.7	71.4	33.3	85.7	33.3	66.7
Cefalexin (Cefamor)	87.5	50.0	100	60.7	33.3	78.6	33.3	33.3
Cefuroxine (Amaxin)	50.0	50.0	66.7	67.9	83.3	57.1	66.7	50.0
Chloramphenicol (Clofencol)	100	100	100	60.7	100	71.4	66.7	66.7
Cotrimoxazole (Septrin)	62.5	100	33.3	28.6	33.3	0.0	33.3	0.0
Cotrimoxazole (B.P (Loxaprim)	50.0	100	33.3	25.0	50.0	42.5	66.7	66.7
Cotrimoxazole (Ranotrim)	25.0	50.0	66.7	14.3	66.7	35.7	33.3	16.7
Cotrimoxazole	50.0	100	100	17.9	66.7	28.6	33.3	50.0
Erythromycin (Erythrokid)	25.0	50.0	66.7	35.7	33.3	42.9	33.3	33.3
Erythromycin (Etocin)	62.5	50.0	100	32.1	66.7	64.3	0.0	50.0
Erythromycin (Throtal)	62.5	100	100	78.6	100	85.7	33.3	100
Flucloxacillin (Floxapen)	12.5	50.0	33.3	32.1	33.3	42.9	33.3	66.7
Metronidazole (Loxagyl)	62.5	100	100	78.6	66.7	71.4	66.7	66.7
Sulfamethoxazole/Trimethoprim (Bactrim)	25.0	100	66.7	17.9	50.0	42.9	33.3	66.7
MAR	28.6 - 61.9	38.1-	57.1 - 100	52.4 - 61.9	38.1 - 76.2	14.3 - 81.0	19.0 - 85.7	28.6 - 81.0

Ant: antibiotics suspensions, *B. cer: Bacillus cereus*, Staph: *Staphylococcus aureus*, Citro: *Citrobacter*, E. coli: *Escherichia coli*, Kleb: *Klebsiella*, Prot: *Proteus*, Salm: *Salmonella*, Shig: *Shigella,* MAR: Multiple Antibiotic Resistance

Table 4: Antibiotic resistance pattern of isolated bacterial flora from urine samples of cows using pediatric oral suspensions (mg/ml)

	% Antibiotic resistance								
Antibiotic suspensions (mg/ml)	***B. cer*** **[2]**	***Staph*** **[1]**	***Citro*** **[5]**	***E. coli*** **[9]**	***Kleb*** **[5]**	***Prot*** **[9]**	***Pseud*** **[1]**	***Salm*** **[2]**	***Shig*** **[5]**
Amoxicillin (Amaxin)	100	100	100	66.7	60.0	66.7	100	50.0	20.0
Amoxicillin / clavunlanic acid (Fleming)	100	0.0	80.0	66.7	80.0	44.4	0.0	100	80.0
Ampicillin (Emcillin)	100	0.0	100	77.8	40.0	55.6	0.0	100	60.0
Ampicillin/ cloxacillin (Emzoclox)	50.0	100	20.0	22.2	40.0	11.1	100	0.0	60.0
Ampicillin / cloxacillin (Jawaclox)	100	0.0	80.0	77.8	80.0	44.4	100	50.0	80.0
Azithromycin (Zithromax)	50.0	0.0	20.0	44.4	20.0	66.7	0.0	0.0	40.0
Cefaclor (Vercef)	0.0	100	20.0	55.6	60.0	33.3	0.0	0.0	20.0
Cefadroxil (Odoxil DS)	100	100	20.0	44.4	60.0	11.1	100	50.0	40.0
Cefalexin (Cefamor)	0.0	0.0	40.0	55.6	80.0	55.6	0.0	50.0	60.0
Cefuroxine (Amaxin)	50.0	0.0	40.0	33.3	60.0	77.8	100	50.0	100
Chloramphenicol (Clofencol)	100	100	60.0	55.6	80.0	44.4	0.0	100	40.0
Cotrimoxazole (Septrin)	0.0	0.0	20.0	44.4	0.0	66.7	0.0	50.0	100
Cotrimoxazole B.P (Loxaprim)	100	100	40.0	55.6	80.0	44.4	0.0	50.0	20.0
Cotrimoxazole (Ranotrim)	50.0	100	40.0	33.3	60.0	22.2	100	0.0	0.0
Cotrimoxazole	100	100	60.0	55.6	80.0	33.3	100	50.0	40.0
Erythromycin (Erythrokid)	50.0	0.0	20.0	44.4	40.0	11.1	100	50.0	60.0
Erythromycin (Etocin)	0.0	100	60.0	55.6	40.0	11.1	100	50.0	60.0
Erythromycin (Throtal)	50.0	100	60.0	77.8	80.0	88.9	100	100	80.0
Flucloxacillin (Floxapen)	100	100	80.0	100	80.0	22.2	100	50.0	60.0
Metronidazole (Loxagyl)	100	0.0	80.0	44.4	60.0	66.7	0.0	50.0	100
Sulfamethoxazole/Trimethoprim (Batrim)	50.0	100	40.0	44.4	60.0	55.6	0.0	50.0	40.0
MAR	57.1 - 61.9	57.1	19.0 - 61.9	28.6 - 81. 0	42.9 - 81.0	23.8 - 61.9	52.4	47.6 - 52.4	23.8 - 71.4

Ant: antibiotics suspensions; *B. cer: Bacillus cereus*; Staph: *Staphylococcus*; Citro: *Citrobacter*; Kleb: *Klebsiella*; Prot: *Proteus*; Salm: *Salmonella*; Shig: *Shigella*; *Pseud: Pseudomonas*; MAR: Multiple Antibiotic Resistance