Small Scale Plastic Anaerobic Biogas Digester Disinfects Ciprofloxacin Resistant *Staphylococcus aureus* (CRSA) from Hospital Wastewater in Cameroon: A Preliminary Report

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Abstract: Disposal of untreated medical and pharmaceutical wastewaters containing antibiotic resistant bugs is frequently discharged onto the environment. Antibiotic resistance is fast becoming one of the most important health problems in both the developing as well as the developed world. The need to embark on this study has been motivated by a number of empirical observations: a retrospective study from January 2012 to April 2014 indicated a growing resistance by Staphylococcus aureus from abscesses, urinary tract and vaginal tract to ciprofloxacin in some hospital and clinics in Cameroon using 500 patients folders which were randomly selected and observed that ciprofloxacin antibiotic resistance was up to 65% with multidrug resistance also observed. Nosocomial Staphylococcal infections are also highly prevalent amongst patients with prolonged hospital stay in Cameroon. Hospital wastewater sampled from 10 health care units in the North West Region of Cameroon was cultured at the Phyto-Biotechnology research laboratory, on Manitol Salt agar and Nutrient agar. Staphylococcus aureus was recovered as the most prevalent bacteria. An antibiotic sensitivity test was carried out using the most frequently prescribed antibiotics in Cameroon for the treatment of staphylococcal infections. We hypothesize that such untreated water used in small-scale agricultural practices in SSA potentially carry antibiotic resistant fluoroquinolones and B-lactams are also being used in human chemotherapeutics. This potentially disseminates resistant microbial strains into humans and thus limits the range of effective antibiotics or human use. Ciprofloxacin resistant Staphylococcus aureus isolates (10,000CFU/ml) from hospital wastewater was re-suspended in peptone water and fed onto a 5 litre plastic digester using poultry and cow manure slurries as starter cultures. After 6 weeks Hydraulic retention time, samples drawn from the digester and cultured yielded no growth for staphylococcus aureus as against control in a round bottom flasks containing CRSA left on bench. Methane gas was collected at the 5th week in a plastic tube for cooking. It was concluded that plastic digesters can potentially disinfects antibiotic resistant Staphylococcus aureus from hospital wastewater while producing biogas for cooking and sterile slurry for gardening.

Keywords: Hospital wastewater, *Staphylococcus aureus*, biodigester, Biogas.

INTRODUCTION

It is estimated that 70% of the population of SSA is involved in of Small scale agricultural practice in which untreated wastewater is applied for irrigation [1,2]. Poor waste management by city councils an industrial wastes and untreated hospital wastewater and leachates from dumped sites, sleeks into and contaminate underground water [1,2,3]. There is a high potential leaching effect into ground and surface water bodies which are the primary source of water for people in SSA for household and irrigation of crops [4,5]. The introduction of antibiotics in the 1940s left the impression that a definitive answer to infectious diseases was found [1,5,6,7]. However, within a few years, microbes had begun to exhibit resistance to antibiotics. For the past half a century, antibiotic resistance has grown prodigiously [1,8]. The most common cause of treatment failure in bacterial infectious diseases is antibiotic resistance (ABR) [9]. Antibiotic resistance has compelled general practitioners and doctors to prescribe more expensive antibiotics, use more toxic antibiotics, increase length of hospital stay and increase cost of healthcare [1]. The resistant strains are common among both the Gram positive and gram negative organisms. The first case of antibiotic resistance was in 1947 where *staphylococcus aureus* was found to develop resistance to penicillin and this lead to the use of another antibiotic called Methicillin. Later, Methicillin-resistant *staphylococcus aureus* was detected in Britain in 1961 and was globally responsible for total cases of blood poisoning [10]. In 1993, it was again discovered that *Escherichia coli* was resistant to Fluoroquinolone variants. Other

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cases reported include penicillin-resistant *enterococcus* in 1983; vancomycin-resistant *enterococcus* in 1987 and the trend continuous until date. Antibiotic resistance can be defined as the ability of the microorganisms to withstand the effect of antibiotic drugs in the concentration that is normally effective [10]. A theory supposes that 'resistance could be due to pre-existing mutants of the resistant strains'. This means that an organism has a natural mechanism to resist antibiotics even without previous contact with the antibiotics. The second theory supposes that 'resistance could be due to the exposure of the bacteria to sub- lethal level of the antibiotics'. Resistance can be intrinsic or acquired [1, 3, 11]. Intrinsic resistance is due to the presence of inherent properties in the bacterial cells [12, 13]. This may occur by either mutation or by acquisition of gene coding for resistance from external source or *via* a transpoon or plasmid. It can as well occur by cross resistance of one strain of micro- organism to another or even more than one organism thus multi-resistance.

The threat to human health posed by antibiotic-resistant bacteria pathogens is of growing concern to medical practice. The emergence of antimicrobial resistance, especially the multidrug resistance to ampicillin, chloramphenicol, and cotrimoxazole, has further complicated the treatment and management of many microbial infections. With increasing hospitali-zations in many hospitals in the developing countries, there will be increasing volumes of untreated waste-water discharged onto the environment. Wastewater treatment technologies are really expensive and unsustainable. There is a need to treat medical wastewater before discharge considering the current threat of emerging and reemerging infectious diseases. In this study, the results of the potential application of Small scale plastic digesters t disinfect hospital waste-water especially wastewater containing ciprofloxacin resistant *Staphylococcus aureus* is presented.

Study Area

The study area selected for this`study is North West, part of Cameroon.

Cameroon is usually described in Africa as Africa in miniature as the country's strategic location displays all geographical, geological features found across Africa. It has two seasons- rainy and dry seasons found across Africa. Cameroon is also part of the Congo forest region. Culturally, there are more than 250 local languages from Tikari, Bantu, Hausa found across Africa. In Cameroon, French and English are spoken which are two dominant languages across Africa. The study was conducted at the Phyto-biotechnology research Foundation and affiliate with the Catholic University of Cameroon. The Catholic University of Cameroon is a recognized Government approved University and the Catholic Church in Cameroon runs some of the best hospitals and healthcare services in the country. The university research division has an institutional ethics review board to handle research ethics. Three wastewater samples (a litre each) from each of the ten hospitals and health centres were collected using well sterilized 1 litre round bottom flasks, properly labelled and transported to the laboratory using standard procedures. This will be done and repeated in two seasons, rainy and dry season in the North West regions of Cameroon. Specimens were collected and cultured on Nutrient agar (NA) MacConkey agar (MCA), Deoxycolate Agar (DCA) and *Salmonella Shigella* Agar (SSA) and Blood Agar (BA agar) to isolate a wide range of microorganisms. Isolates from the primary cultures were subcultured into fresh peptone medium to obtain pure isolates. Pure isolates were inoculated onto nutrient agar slant and stored at 5°C for further characterization and identification [1, 14].

Characterization and Identification

Pure isolates obtained on nutrient agar were identified and characterized based on colonial morphology, cultural characteristics, Gram stain reaction, and biochemical tests. *Staphylococcus aureus* was frequently isolated and confirmed using positive catalase and coagulase tests on manitol salt agar [1,14].

Antibiotic Sensitivity Testing

An antibiogram tests was done using the commonly prescribed antibiotics for treating infections in Cameroon. Antibiotic sensitivity testing was done using Kirby-Bauer method on Mueller Hinton Agar. A 3.8 g of this agar was dispensed into a sterile conical flask. One hundred millilitres (100 mL) of distilled water was poured into the flask and stirred to dissolve the agar. The mixture was autoclaved and then poured into petri dishes. On gelling, commercially prepared antibiotic sensitivity disk was introduced using sterile forceps and then incubated for 24 hours at 37°C [1,15,16].

During plate reading, it was observed that all the bacterial isolates had less than 40% sensitivity to the


Plate 1: The anaerobic digester.

antibiotics used. Ciprofloxacin, one of the most prescribed fluoroquinolone antibiotics had no inhibition of staphylococcus isolates. For the purpose of disinfection studies, we focused on using ciprofloxacin resistant *Staphylococcus aureus* as an index to study the efficacy of the small scale plastic digester.

DIGESTER DESIGN AND CONSTRUCTION

The anaerobic digester used in this study was constructed using a longitudinal wooden trough (triangular in shape) the dimension of the trough were: Length of top and bottom, 1.8m and 1.26m respectively while bottom width and top width, 0.45m and 0.8m respectively [17,18]. Equally, the depth and slope of the trough measured 0.3m and 0.4m respectively. At both ends of the trough, plastic pipes were initially cut to specifications using the Hawk saw and edges smoothened using the half round file. Fairly thick plastic polyethene sheet with opening at both ends was first laid down as bedding in the trough and then folded two-step wise at both ends into two layers and fed in gently through the pipes [1, 5, 18, 19, 20]. The edges of the Polyethene were wrapped round the mouth/opening of the pipes and fastened in position with a rubber band. The polyethene sheet was thoroughly checked for holes so as to avoid any leakage when the process was fully operational. Plate **1** the Anaerobic digester.

Plate 2: Digester also produces gas.

Slurry Preparation

Two ten litre of buckets full of cow manure was used. This was used as starter culture for the small scale digester. The microbial content of the cow manure was analysed at the Phytobiotechnology Research laboratory and content known. Slurry was made by mixing one part of the cow liter in two parts of water. The microbial content of the water was also analysed and known. This was thoroughly mixed by stirring continuously for some few minutes. All in all, about 100 litres of slurry or there about was fed into the digester. As effluents were added to the digester, excess water drained off at the outlet. The digester was actually filled to the brim with space provision for gas to collect.

Slurry Sample Collection for Microbial Analysis

When the slurry was fully made, three samples (10mls each) were collected aseptically using sterile stainless steel spatula into sterile test tubes. The test tubes were sealed with cotton wool and then transported to Microbiology bench of the Phytobiotechnology Research laboratories for analysis. Similarly, the well water that was used in making the slurry; three samples of it were taken aseptically in the same way as described earlier for a comparative microbial analysis. Following incubation at 24 and 96 hours respectively for bacteria and fungi respectively, the plates were read off. The cultural characteristics such as shape of colonies, colour etc was observed macroscopically and recorded. Then discrete bacterial colonies from each plate were gram stained and observed microscopically at 1500 magnification [1].

Microbial Analysis of Treated Hospital Wastewater Slurry

Anaerobic digestion of the raw slurry was allowed for six weeks at mesophilic temperature. Following this

Table 1: Bacteriological Analysis Results of Raw Cow Manure Slurries at 10^{-3} Dilution

	Total Aerobic Mesophilic Counts	Coliform Counts	E. Coli Counts	SS Counts
Raw Slurry	TNTC	TNTC	TNTC	TNTC
Treated Slurry	140 CFU/ml	110 CFU/ml	82 CFU/ml	Nil

TNTC → Too numerous to count.
CFU/ml → Colony forming unit per mililitre.
Nil → No colony isolated.
E. coli → Escherichia coli.
SS → Salmonella and shigella.
Pa-------- Pseudomonas aeruginosa.

Table 2: Bacterial Analysis Result of well Water and Raw Slurries Placed on Bench for 6 Weeks at 10^{-3}

Total Aerobic Mesophilic Counts	Coli form Counts	E. Coli Counts	SS Counts	Ciprofloxacin resistant Staphylococcus aureus (CRSA) Mean values recorded
At the beginning of experiment TNTC	TNTC	TNTC	Nil	10,000CFU/Ml peptone water
After 6 weeks of biodigestion TNTC	5.2×10^4	3.7×10^4	Nil	No Colony isolated

TNTC → Too numerous to count.
Nil → No isolates.

treatment and as the gas start to fill in the gas collector, three samples of the slurry now (treated slurry) were aseptically collected and analysis followed the same procedure as earlier described. Similarly, it was at this time that the well water samples which were initially used in making the slurry and kept at room temperature (37^{oc}) in the laboratory for five weeks were then microbiologically analyzed in the same procedure.

DISCUSSION

Antibiotic resistance is now being recognized as a major factor determining morbidity, mortality and cost in the medical arena. Various strategies like infection prevention, infection eradication, and containment of resistant species and optimization of antibiotic utilization are generally considered. In this study as seen in Tables **1** and **2**, the initial microbial counts in the hospital wastewater are very uncountable. Many organisms that are of public health significance were isolated from the wastewater from the health units in the study area in Cameroon. Bacterial isolates such as *Pseudomonas aeruginosa*, many strains of E coli and *Staphylococus aureus* were isolated. In a previous study to disinfect cow dung pathogens [2, 3,4] reported a similar spectrum of pathogens. In this study, *staphylococcus aureus* was isolated from all the wastewater samples from all the health centres. Antibiotic sensistivity tests revealed that most of the organisms isolated from hospital wastewater were insensitive to currently used antibiotics *in vitro*. This observation has not been earlier reported. For this reason, particular attention was paid on the effect of anaerobic digestion technology on disinfection of antibiotic resistant bugs. The initial counts of CRSA was 100 000 CFU/Ml and after a 6 week anaerobic digestion residence time, no colony of *staphylococcus aureus* was isolated. Anaerobic digestion offers a good alternative for the treatment and disposal of animal waste. Anaerobic bioconversion technology has been in existence for more than a hundred years but the adoption of this technology in Africa could bring a whole range of benefits especially in the management of hospital wastewater. More often the large scale Chinese dome shape digesters are used and are extensively studied. The cost, however, play a prohibitive role for adoption in Africa. In this study, the choice of small scale plastic digester is critical for adoption and such an extra benefit such disinfection of wastewater is an important step toward adoption. Little work has been done in determining the fate of pathogens in a plastic type digester especially under tropical conditions [1, 8, 12, 19, 20]. It was in this regard that the efficacy of disinfection was monitored for 6 weeks weeks through culture tests.

CONCLUSION

The conclusion is drawn that small scale plastic digester can disinfect not just pathogens in wastewater but drug resistant bugs. It is also concluded that medical wastewater can be treated using low cost

ecologically sound technologies by employing plastic digesters.

RECOMMENDATION

Since this was a preliminary study geared toward checking out the disinfection potential of small scale digester on antibiotic resistant bugs, there is a need to carry out more bench studies to further confirm and strengthen the findings herein. Additionally, studies on the potentials for scale up tha can treat large volumes of hospital wastewater are absolutely necessary.

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