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Original Research Article

POTENCY EVALUATION OF A FORMULATED NOVEL DISINFECTANT ON PATHOGENIC BACTERIA ISOLATED FROM AUTOMATED TELLER MACHINES (ATMs) IN BAYELSA STATE, NIGER DELTA, NIGERIA.

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Abstract

An increasing number of people has used the Automated Teller Machine (ATMs) by the year, but less was known about their bacteria colonization status. Do these machines harbor pathogenic bacteria? This work was carried out between March and November 2015 to isolate the pathogenic bacteria associated with ATMs and to evaluate the potency of the formulated novel (Darol) and commonly sold disinfectants (Izal, Domitol) on these bacteria isolates. Samples were collected from thirty-two (32) different Automated Teller Machines located in different banks, within Yenagoa metropolis, with wet sterile swabs and cultured, isolated and identified in the laboratory using selective media and microbiological standard procedures respectively. The bacteria suspension of pure isolates were standardized with 0.5 Mc Farland Turbidity Standard and subjected to antimicrobial susceptibility testing of the disinfectants at different concentrations (2% 4% 6% 8% 10% and 12%) using cup plate method. Seventy-nine (79) bacteria strains which include *Staphylococcus aureus*, 37(46%); *Klebsiella* sp., 19 (24%); *Pseudomonas* 6 (7.5%) and *Bacillus* 6 (7.5%) were isolated and identified. Statistical analysis showed no significant difference in the potency of the test disinfectants ($p<0.05$) at different concentrations except at 2% between Darol and others for *Klebsiella* and *Pseudomonas* species. In conclusion, findings have shown that the potency of the test disinfectants increases with increase in concentrations and Darol mean value 75.5 (43.1%) was most potent, followed by Damitol mean value 54.1 (31%) and the least was Izal mean value 45.4 (26%).

Keywords: Automated Teller Machine (ATM), Formulated Novel disinfectant, Potency evaluation, Bacteria

Introduction

An Automated Teller Machines or Automatic Teller Machine (ATM) is a computerized telecommunications device that enables the clients of a financial institution to perform financial transactions without the need for a cashier, human clerk or bank teller. ATMs are known by various other names including ATM machine, automated banking machine, cash dispenser

and various regional variants derived from trademarks on ATM systems held by particular banks (Automatic teller, 2012**).** Automated teller machines (ATMs) are the longest standing and most widely used a form of the computer driven public technology (Hone et al., 1998), with an estimated over 2.4 million units in use (Anonymous 2011) since their invention and use in the late 1960s. Working as a data terminal communicating through a host processor which links all other such machines operated by a bank across a wide area network, it makes a cash withdrawal and other services available to the account – holder more convenient. A typical usage of the machine involves slotting a card into a recipient hole and following on-screen instructions by punching the keys of the metallic keypads to enter secret codes and commands; thus instructing the machine as to the kind of transaction one requires (Anonymous 2011). There is no restriction as to who has access to the facility, and no guidelines to ensure hygienic usage, but like all surfaces, microbial colonization of these metallic keypads are eminent, particularly so when there is no proper cleaning regimen in place for most of these facilities. Such colonization and their subsequent biofilm formation have been the theme of research by several investigators (Hood and Zottola 1997; Sharma and Anand *et al.*, 2002). Mbajiuka *et al*. (2014) stated that human beings have a marked tendency to pick up microorganisms from the environmental objects and the hand has been shown to play a role in the transmission of the organism. Colonization of objects by pathogenic organisms has been reported as a potential vehicle for their transmission (Famurewa and David *et al.*, 2009). Likewise in Mbajiuka *et al.* (2014)'s and Dogan *et al.* (2008)'s report which stated that cell phones of patients, visitors, health workers and computer mice, keypads respectively in the hospitals and in education institutes carried multiple drugs – resistant hospital pathogens including *Acinetobacter s*pp, *Staphylococcus aureus* and extended – spectrum B-lactamase, ESBL- positive Enterobacteriaceae, hence they suggested frequent disinfection of mobile phones and these devices to reduce bacteria reservoir on them. Furthermore, microorganisms found on contaminated surfaces have been shown to persist on environmental surfaces for a varying period of time ranging from hours to months (French *et al.*, 2004). Hence cross infection of microorganisms between environmental surfaces and a host equally been established (Hardy *et al*., 2006). These organisms can cause serious infections when they gain entrance into the human body. Bacteria that can cause severe gastroenteritis have been found on ATM keypads (Fraser, 2009). Disinfectant describes a product applied directly to the inanimate object. It destroys or irreversibly inactivates most pathogenic organisms but not usually spores (Mabel *et al.*, 2014). Disinfecting agents are registered by Environmental Protection Agency (EPA) as antimicrobial pesticides and are substances used to control, prevent or destroy harmful microorganisms on the inanimate objects. Disinfection protocols, when implemented correctly, can be cost effective means of reducing pathogenic organisms and are an important step in any biological risk management program. Prevention of disease is typically easier and more cost – effective than addressing an outbreak situation. Therefore development and implementation of a step – by -step disinfection protocol for the control and prevention of infectious diseases have become essential in public facilities such as the ATMs. This work is thus carried out to isolate the pathogenic bacteria associated with Automated Teller Machines (ATMs) and do the comparative evaluation of the potency of formulated novel and commonly sold and use disinfectants on the bacteria isolated to enable us as health professionals recommend the more potent disinfectant for routine disinfection.

Materials and Methods

Study Areas

This study was carried out in Yenagoa metropolis, the capital city of Bayelsa state, the state situated between Delta and Rivers states, the southern part of Nigeria.

Sample Collection

Samples from thirty-two (32) Automated Teller Machines (ATMs) from different banks and ATMs located within the Federal Medical Centre Yenagoa were used for this study. The names of the banks are Access, Diamond, UBA, Stanbic IBTC, Keystone and Fidelity banks. Permission was sought from the management of the banks to collect samples from their devices.

Materials and Media used

Sterile swab sticks, sterile distilled water, Mentholated spirit (Moko), sterile universal bottles, Syringes (2ml-5ml), Cotton wool (Dr Whiter) Petri dishes, Wire loop, autoclave, dryer, Hot air oven, foil paper, bijou bottles, water bath, beakers, plasma, incubator, colony counter and Automated Teller Machines (ATMs).

Commercial and Formulated novel disinfectants and their Constituents

Izal: contains Carbonated Cresol as active constituent; Domitol: contains Lysol and Phenol as active ingredient; Formulated novel disinfectant (Darol) contains Caustic Potash 13.6g; Eucalyptus oil 63.0g; Ethanol 95% 200ml; Terpineol 100ml; Oleic acid 7.5ml; Chloroxylenol 50g and Purified water 60ml

Media

Growth media used for the isolation and characterization of the pathogens include Nutrient agar, Eosin Methylene Blue (EMB), Mac Conkey agar, Mannitol Salt Agar (MSA). These media were prepared according to manufacturer's instructions and were steam sterilized at 121^{o}C for 15minutes.

Isolation of Pathogenic Bacteria from ATMs

Sterile swab sticks were slightly moistened with sterile distilled water and were used to swab the metallic keypads and screen- pads of the various AT machines, and immediately transported to the pharmaceutical microbiology laboratory for culture. The streak plate method was used and this was done in triplicate onto sterile selective media mentioned above. The sets of plates were then incubated at 37^{o}C for 24hrs. Resulting pure colonies were transferred onto nutrient agar for subsequent characterization and identification.

Characterization and Identification

Pure cultures of bacteria isolated were characterized and identified on the basis of their cultural, morphological and biochemical properties and by reference to Bergey's Manual of Determinative Bacteriology (Cowan and Steel's Manual for the identification of Medical bacteria, Barron and Feltham 1999; Medical Microbiology (Geo *et al*., 2001). The pathogenic bacteria isolated include *Staphylococcus aureus*, *Klebsiella* species, *Pseudomonas aeruginosa* and *Bacillus* species.

Preparation of bacteria Suspension

The pathogenic bacteria isolated were grown in Nutrient broth oxide overnight. Culture was centrifuged at 512g (sigma model 3k-1) for 10mm and resulting cell pellets resuspended in 0.1% peptone

Disinfectants used and their preparations

The test disinfectants include the commercial and formulated novel disinfectants which include Izal, Domitol and Darol respectively. Thirteen point six gram (13.6g) of caustic potash was weighed on an analytical balance and transferred into a stainless steel vessel. 15ml of distilled water was then measured and used to dissolve the 13gof caustic potash in the stainless steel. 63g of eucalyptus oil was weighed on an analytical balance and dissolved in 63ml of 95% ethanol mixed properly and allowed to stand for one hour. After about an hour 7.5ml of Oleic acid was then added to the eucalyptus oil- ethanol mixture. Step B: 50g of Chloroxylenol was

weighed on an analytical balance and mixed with the remaining part of 95% ethanol (137ml). 75ml of terpineol oil was then added to the mixture (chloroxylenol and ethanol). Step C: Step B product was then poured into step A product. The remaining 45ml of distilled water was then added and finally made up to one liter of distilled water.

Preparation of test Disinfectants

Disinfectants Darol (formulated), Domitol and Izal were diluted in sterile distilled water prior to use at concentrations of 2%, 4%, 6%, 8%, 10% and 12%. The products and the recipes used in preparations were obtained from a chemical shop. Cup plate method was used and the disinfectants were prepared thus: 30ml of Nutrient agar was prepared according to manufacturer's instruction; this was then poured into a sterile petri dish and was allowed to set. 0.3ml of the microbial standardized suspension was inoculated using spread plate method on the agar plate. Cups were then made on the sterile nutrient agar using a sterile cork borer. The bottom of the cups was sealed with molten nutrient agar and allowed to gel. These were then labeled according to the different concentrations of the test disinfectants. 0.5ml of the different concentrations of the disinfectant were then introduced into the cups and allowed to stand for some minutes and incubated at 37^{o}C for 24hours. The zones of inhibition on the incubated plates were measured and recorded appropriately using a millimeter rule.

Evaluation of Disinfectant Potency (Phenol Coefficient test Rideal – Walker co- efficient method)

An overnight culture of *Staphylococcus aureus* was prepared in a nutrient broth and poured into sterile test tubes. Five (5) empty sterile test tubes were provided and labeled A B C D and E. 5ml of phenol was then dispensed into the empty sterile test tubes in the following concentrations 1/70, 1/80, 1/90, 1/100, and 1/110. The test tubes containing nutrient broth were placed in 5 rows of 4 in each means 20 tubes altogether. The tubes were labeled as follows: A1 B1 CI D1 and E1; A2 B2 C2 D2 and E2; A3 B3 C3 D3 and E3; A4 B4 C4 D4 and E4 respectively such that empty tube ABCDE has 4 tubes corresponding to it. 0.2ml of the culture was then dispensed into the test tube labeled A, using a pipette, at zero time. At 30 seconds interval tube B was then inoculated with 0.2ml of the broth culture. This procedure was repeated at 30 seconds intervals for tubes C, D, and E. A loop full taken from tube A was used to inoculate tube A1, also a loop full taken from tube B was used to inoculate tube B1 and repeated with tubes C, D, E and C1 D1 E1 and E4, each inoculation was done at 30 seconds interval. The entire procedure was then repeated using tubes labeled P, Q, R, S, T containing the novel disinfectant and another test disinfectant in the following concentrations: 1/500, 1/700, 1/900, 1/1100, 1/1300. All tubes were incubated at 37^{o}C for 2 – 4 days and examine for growth and the result recorded.

Results

A total of seventy-nine (79) microorganisms were isolated from the ATMs located in different banks and public places within Yenagoa metropolis, Bayelsa state, Nigeria. These organisms include *Staphylococcus aureus* 37 (46), *Klebsiella* species 17 (21%), *Pseudomonas aeruginosa* 19 (24%) and *Bacillus* species 6 (7.5%) Figure 1.0 depicted the frequency of bacteria isolated from the different Automated Teller Machine examined. *Staphylococcus* was having the highest frequency of 37 followed by *Pseudomonas* 19 then *Klebsiella* 17 and *Bacillus* was having the least of 6. Figure 2.0 showed the graph of the mean values of the zone of inhibition; revealing the efficacy of different disinfectant concentrations on *Staphylococcus aureus*. The formulated novel disinfectant (Darol) had the highest zones of inhibitions at different concentrations used on all the test microbial

isolates. Figure 3.0 depicted the graph of the activity of the different concentrations of the test disinfectants on *Klebsiella* sp. isolates. Darol demonstrated the highest potency on *Klebsiella* sp. when compared with other two test disinfectants. Figure 4.0 showed the graph of the effects of disinfectants concentrations on *Pseudomonas aeruginosa*; Darol was the most potent followed by Domitol and the least potent was Izal at different concentrations used on this organism. Figure 5.0 depicted the bar chart of the different concentrations of the disinfectants on different concentrations of the disinfectants on *Bacillus* sp. Darol disinfectant was the most potent followed by Izal and the least was Domitol at 12% concentration, but Domitol was more potent than Izal at 10% concentration.

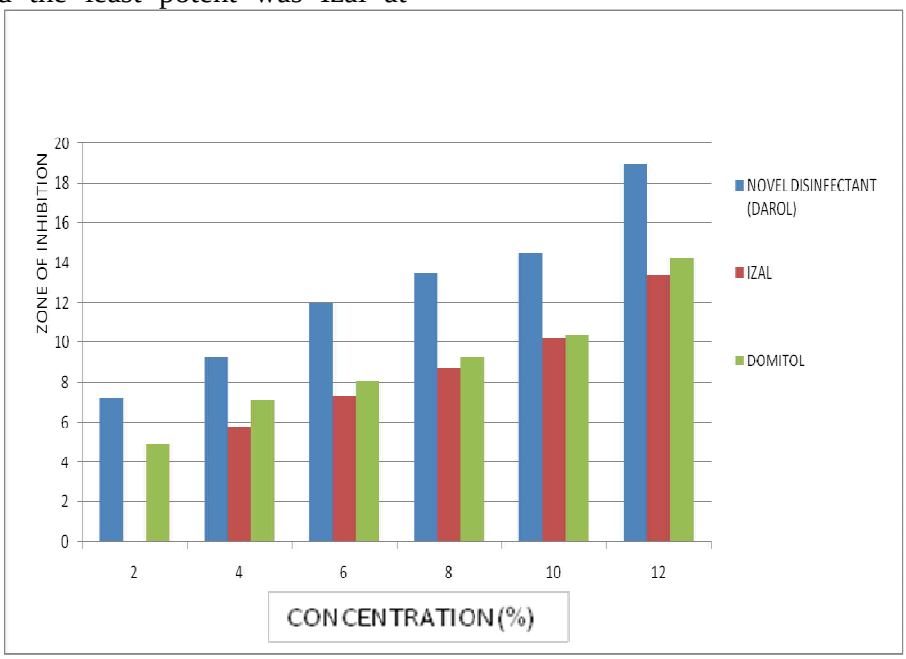

Figure 1.0: A Bar Chart of the Activity of Different Concentrations of the Disinfectants on *Staphylococcus aureus*

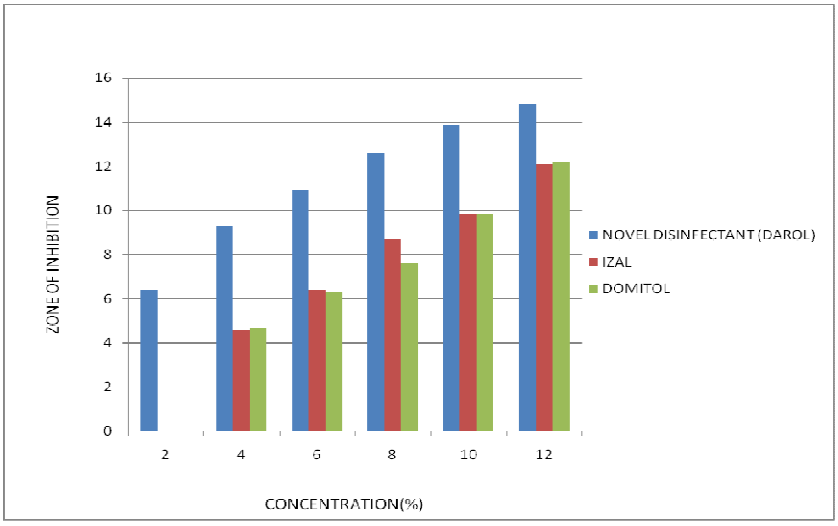

Figure 2.0: A Bar Chart of the Activity of Different Concentrations of the Disinfectants on *Klebsiella* species

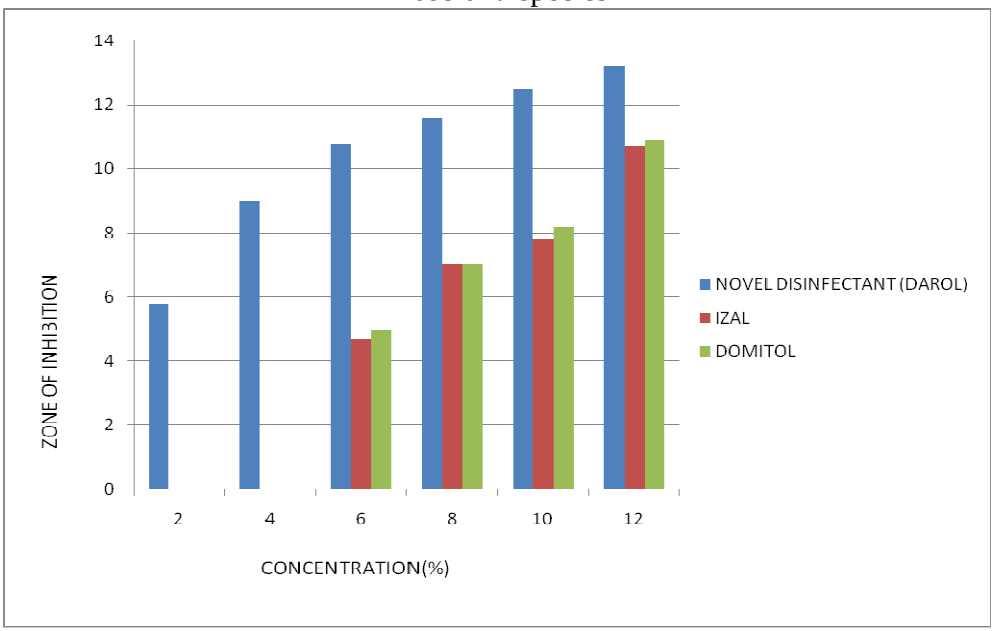

Figure3. A Bar Chart of the Activity of Different concentrations of the Disinfectants on *Pseudomonas*

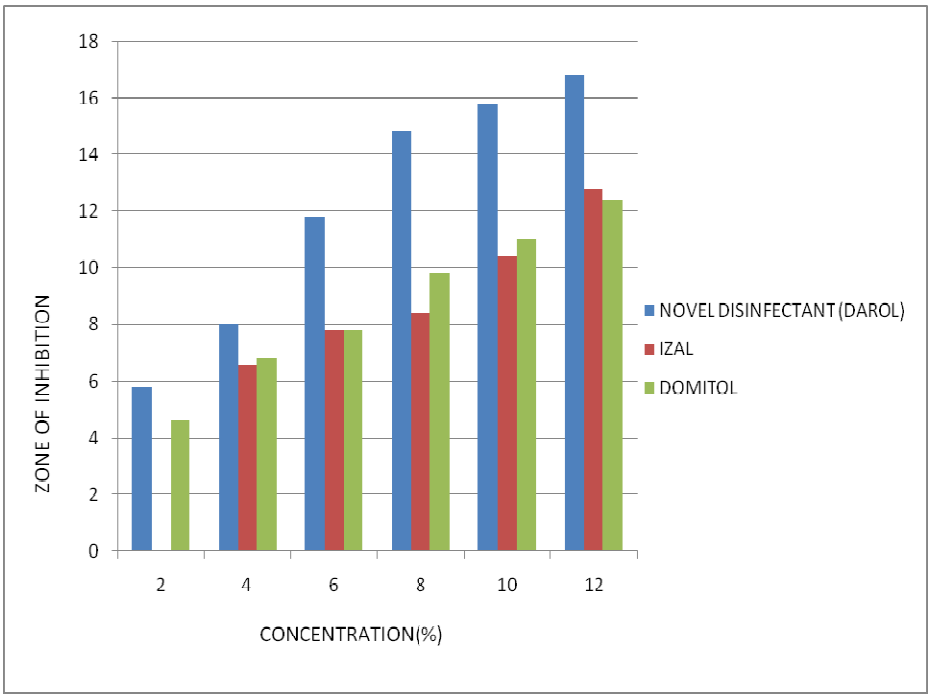

Figure 4.0: A Bar Chart of the Activity of the Different Concentrations of the Disinfectant on *Bacillus* species

Discussion

A total of seventy-nine (79) bacteria were isolated from the ATM located in different banks and public places within Yenagoa metropolis, Bayelsa state, Nigeria. The organisms which include *Staphylococcus*

aureus 37 (46%), *Klebsiella* sp. 17 (21%), *Pseudomonas aeruginosa* 19 (24%) and *Bacillus* sp. 6 (7.5%) were subjected to disinfectants (at different concentrations) susceptibility testing using cup border method. The zone of inhibition was measured and the mean values calculated; this was represented graphically as shown in figure 2.0 to 5.0. The pathogenic bacteria isolated in this study are considered to be of high public health risk. The predominant organism isolated was *Staphylococcus aureus* and the least was *Bacillus* **s**p.; this is in concurrent with the work of Kluytmans *et al.* (1997); Anastasiades *et al.* (2009); Abban and Tano-Debrah (2011) and Ogston *et al.* (1984) which stated that 20% of human population are long-term carrier of Staphylococcus aureus that can be found as part of the skin flora and prevalent on computer keyboards and mouse**. (**Abban and Tano-Debrah 2011) and Ogston *et al.* (1984) **r**eported that the ATMs are infected with *Staphylococcus aureus, Pseudomonas sp. Klebsiella sp. Bacillus* sp. and other well documented pathogenic bacteria. *Staphylococcus aureus* is a common cause of skin infections, respiratory disease eg (sinusitis) and food poisoning. These findings have shown that the prepared disinfectant (Darol) with a phenol co-efficient of 8.75 demonstrated the highest potency at different concentrations on all the microbial isolates; followed by Domitol and Izal. The potency of Darol on these isolated organisms can be attributed to its chemical constituents, one of them is Chloroxylenol; a broad spectrum antimicrobial, chemical compound used to control bacteria, algae, fungi, and virus, it is used in the hospitals and household for disinfection and sanitation (Batiz-Lazo and Reid 2008; Hone *et al.*, 2008). Another component of Darol is ethanol – a common antibacterial agent which kills microorganisms by denaturing their proteins and dissolving their lipids and is effective against most bacteria, fungi and many viruses (Mehmet *et al.*, 2013**)**. Also caustic potash (Potassium hydroxide of which the corrosive properties of potassium hydroxide make it a useful ingredient in agent and preparations that can clean and disinfect surfaces and materials Rompp Chemie- Lexikon) At 12% and 10% concentrations Darol were more potent on *Staphylococcus aureus* with a mean value of 19 $ 14.5, standard deviation 0.8 &1.9 than *Klebsiella* sp. mean value of 14.8 and 13.9 standard deviations 0.75 &1.10; *Bacillus* sp. mean value 16.8 and 15.8, standard deviation 1.94 &1.30; *Pseudomonas s*p. mean value 13.2 &12.5, standard deviation of 0.79 &0.97. These findings had shown that *Pseudomonas* sp. were more resistant to Darol than other microbial isolates at both 12% and 10% concentrations, followed by *Klebsiella s*p. while *Staphylococcus aureus and Bacillus* sp. were more susceptible than *Klebsiella* sp. and *Pseudomonas s*p.; this could be attributed to the fatty materials such as lipopolysaccharide and lipolipids on their cell walls being gram negative bacteria and contribute to their resistance to antimicrobial agents such as Darol used in this work.

Conclusion

On the basis of these findings, Darol, the novel disinfectant is advised for disinfecting the Automated Teller Machines by the Banks especially in Nigeria and some other developing countries where everyone needs to touch the machines before getting out the money. Likewise, the manufacturers of the other two disinfectants (Domotol and Izal) are advised to incorporate (as part of the constituents) Chloroxylenol into their product for more effectiveness.

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