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THE SANITISING EFFICIENCY OF DIFFERENT DISINFECTANTS ON *SALMONELLA* ISOLATES IN PORT HARCOURT ABATTOIRS

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ABSTRACT

This study examines the bactericidal efficiency of common disinfectants parachlormetaxylenol (dettol) ; savlon, purit, hypochlorite solution (jik) acetone, methanol, ethanol and phenol on Salmonella species isolated from five different abattoirs in Port Harcourt metropolis using Agar dilution method from January 2005 to June 2006. The abattoirs sampled were located at Agip, Trans Amadi lndustrial layout, Woji, Rumuodara and Rumuokoro. Salmonella species were isolated from the slaughter floor in these study abattoirs using a selective medium Salmonella Shigella agar medium. Salmonella suspension was prepared and standardized with 0.5 Mac Farland turbidity standard of standardization. The efficacy of the test disinfectants at different concentrations of 10%, 20%,40% and 70% was tested on this salmonella isolates. Statistical analysis showed no significant difference in the efficacy of the disinfectants used ($p<0.05$) at different concentrations, except between dettol and other test disinfectants. In conclusion this study has shown that at 10% concentration, Salmonella species isolated were resistant to jik (sodium hypochlorite) and susceptible to dettol, purit, and savlon with 2 to 0 colonies counted on the petri plates. Though dettol was more effective on microorganisms than purit and savlon at 10% concentration after a contact time of 10 minutes and 24hrs incubation.

Keywords: Disinfectants efficacy, *Salmonella*, Abattoirs floor, Environment, Food safety.

INTRODUCTION

A slaughter house (abattoir) is an industrial facility where animals are processed for consumption as food products. In the United States, about ten billion animals are slaughtered every year in 5,700 slaughter houses (Williams *et al*., 2007). In 2007, 28.1 billon pounds of beef were consumed in the United State alone (U.S. Beef and Cattle Industry, 2007). It was also observed that in Canada, 650 million animals are killed annually and in European Union the animal figure is 300 million cattle, sheeps and pigs (Hershaft, 2008). Inspite of efforts at surveillance, incidents of salmonella infection are grossly unreported throughout the world (Blaha, 2000). The author observed that in England and Wales, human salmonellosis cases double between 1966 and 1971 mainly due to a three fold increase in the occurrence of salmonella serotypes other than *Salmonella typhimurium*- In United State of America, two million human cases and 500 deaths, and related economic looses of 100 million dollars annually are attributed to salmonella infection. However in Canada according to Friedman *et al*. (2000)'s report which stated that over 500,000 persons are afflicted per year with salmonella infections. During the summer of 1974, outbreaks of food poisoning caused by *Salmonella infantis* were reported in South Wales, the Midlands and the West Country. Investigation suggested that the vehicles of infection were cooked meats and pies supplied by one food factory. In view of the high rates of salmonella contamination on meat generally, it is not surprising to find meat so often the cause of salmonellosis. Miller *et al*. (2000) found in Scotland that meat was responsible for 224 outbreak affecting 2,245 people between 1980 and 1985; this represented 52% of the total number of

salmonellosis causes where the food could be identified. Again Dupont (2000) found that between 1959 and 1985 in England and Wales some 436 of family and general outbreaks of salmonella food poisoning could be attributed to the consumption of meat products. PHLS Report (1989) discovered that there has been an enormous increase in salmonellosis in many parts of the world, even similar trends have been reported in many other countries including France, West Germany and Italy. In the north-east of the United States there was a six fold increase in reported salmonella infection between 1976 and 1986 and this was attributed to eggs or egg containing foods (St Louis *et al*., 1988). In Nigeria the Statistical analysis in the medical laboratories and various hospitals and health care delivery centres has revealed the rate people are suffering from typhoid fever, the disease caused by *Salmonella typhi*, up till date, and this could be attributed to consumption of contaminated food products and water.

Typhoid fever is caused by *Salmonella typhi* or *Salmonella paratyphi* member of the genus salmonella described and studied. Typhoid is the most severe of the diseases caused by salmonella (Downes and Ido, 2001). After a length incubation period of 7 to 21 days, the disease sets in with a general feeling of malaise and during the first week the body temperature steadily rises. About the seventh to tenth day a rash appears and during the second week the fever is at its highest. Death may occur from the severity of the disease at this stage but in the less severe cases there is a gradual improvement in the third or fourth week. *Salmonella typhi* is excreted in the faeces in large numbers during the illness and as with salmonellosis; a carrier problem exist with patients who have recovered and with symptomless excreters.

Disinfectants are chemicals used to inhibit or prevent the growth of microbes on inanimate objects. Usually disinfectant are ‘’cidal’’ in that they kill the susceptible potential pathogenic agents (Rossoni and Gaylarde, 2000). The selection of a disinfectant should be based on the job they are expected to do not necessary on a sales pitch or on what one has always used. Disinfectant used in hospitals, industries and laboratory must be tested periodically to ascertain its potency validation is defined as ‘’establishing documented evidence that a disinfection process will consistently remove or inactivate known or possible pathogens from inanimate objects. Disinfectant can be used to sanitize abattoirs’ (slaughter) floor to reduce *Salmonella* species contaminants before animals are placed there for slaughter. This is to avoid contamination of these microbes on the meat carcasses to promote food safety. The focus on safer foods and longer shelf-life has led to more frequent use of chemical disinfectants (Langsrud et al.,2003). The food safety and inspection service (FSIS), an agency of the United States Department of Agriculture (USDA), is the public health agency responsible for ensuring that the nation’s commercial supply of meat, poultry, and egg products is safe, wholesome, and correctly labeled and packaged (Food Safety and Inspection Service 2010); Center for Disease Control and Prevention, 2008). The significant corporate consolidation of global food production has created a food system and environment for processing the values quantity over quality. According to the center for Disease Control and Prevention, 325,000 people are hospitalized for food related illness and 5,200 die in that same time period. Only a small percentage of those illnesses and deaths are a result of known pathogens. Mundell (2008)’s investigation stated that some of the most common infections are the result of three kinds of bacteria: *Campylobacter, Salmonella* and *Escherichia coli* 0157:H7.

The abattoir environment in Port Harcourt Rivers State in Nigeria are highly contaminated with some microorganisms, but the scope of this study is to cultivate *Salmonella* species, the causative organism of typhoid fever from the slaughter floor. This study is thus undertaken to cultivate *Salmonella* species from the floors where cows are being slaughtered and subject it on different concentrations of common disinfectants in order to get a suitable or the most potent disinfectants among the test ones that would be used to sanitize these floors before animals are placed for slaughter. This is to prevent the consumption of contaminated meat with salmonella and to reduce the spread of typhoid fever among the people.

MATERIALS AND METHODS

Samples from the slaughter floor were collected with sterile cotton swabs. The swabs were held in sterile forceps and the sampled surfaces were swabbed 10times from the top to the bottom. These

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samples (from the slaughter floor were randomly collected every other week for a period of one and a half year starting from January 2005 to June 2006). The samples were taken from five different abattoirs slaughter floors in Port Harcourt metropolis, Rivers State, Nigeria, these abattoirs include Rumuodara, Rumuokoro, Woji, Trans Amadi and Agip located at the West south, Southern, North west, Western and Eastern part of Port Harcourt City respectively. The samples were taken to the laboratory immediately and cultured.

Test Materials and Reagents

A selective medium Salmonella - Shigella Agar was used for the cultivation of *Salmonella* species. The following equipment were used – Microscope, microscopy slides, cover slips, cotton wool, sterile swab sticks. Reagents used include physiological saline, gram stains - safranin, lugols lodine, differential alcohol and crystal violet, oxidase reagents, catalase. hydrogen peroxide, dextrose, sucrose, mannitol. Plasticine, peptone water and kovac's reagent (lso amyl alcohol, P-dimethyl amino benzaldehyde and concentrated Hydrochloric acid), Methyl red and Potassium hydroxide. Agar media included Urea agar slant, Simmon Citrate agar, gelatin liquefaction medium, Nitrate broth; Reagents included IN Hydrochloric acid and 0.2% solution of Sulphanilamide, 0.1% N- naphtylethylene diamine hydrochloride.

Isolation of the Bacteria

The spread plate method was used, serial dilutions of the swab sample was made by adding 1.0ml of phosphate buffer into the swab container and mixed thoroughly. From this, serial dilution was carried by adding 9ml into sterile distilled water to give 10^{-1},10^{-2},10^{-3},10^{-4} and so on. 0.1ml was plated in triplicate onto sterile selective medium mentioned above. The sets of plates were then incubated at 37 ^{0}C. Resulting pure colonies were transferred onto nutrient agar for subsequent characterization and identification.

Characterisation 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, (Holt et al., 1994) and Cowan and Steel's Manual for the Identification of Medical bacteria (Barrow and Feltham, 1993). The test bacteria used are *Salmonella* species.

Preparation of Bacteria Suspension

Organisms used in this experiments were, *Salmonella* species. Bacteria were grown in Nutrient Agar Broth oxoid overnight. Cultures were centrifuged at 512g (sigma model 3k-1) for 10mm and resulting cell pellets resuspended in 0.1% peptone.

Preparation of Test Disinfectants

Disinfectants parachlorometaxylenol (PCMX) (dettol), savlon, purit and Sodium hypochlorite (jik) acetone, phenol, methanol, and ethanol were diluted in sterile distilled water prior to use to 10% and 20% 40%, and 70% concentrations these products were obtained from Reckit Benckiser Nigeria Limited Lagos, Nigeria, Johnson & Johnson, Lagos; CAP Plc (Chemical and Allied Products), Lagos respectively and used without further purification. The pH of the mixtures was controlled by the addition of HCL or NaOH as appropriate.

Composition of Test Disinfectants

Dettol's composition include Chloroxylenol B.P.C. 4.8%w/v, Oleum Pini Aromaticum 90% v/w, Denatured spirits 11.3%w/v, Sapo vegetalis 5.8%w/v, Saccharum Ustumqs, aqua ad 100vol. . Purit contains Chlorhexidine Gluconate B.P. 0.3%w/v and Centrimide B.p. 3.0%w/v. Savlon constitutes n-Propyl alcohol 2.84% m/v, preservative Chlorhexidine gluconate 0.3g and Centrimide 3.0g all in 100ml. Hypochlorite, methanol, ethanol, phenol, and acetone contain sodium hypochlorite as an active ingredient; methane & alcohol; ethane & alcohol; phenyl benzene & ethanol and acetic acid & ketone respectively.

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Suspension Test (Traditional Plate Count Method)

Approximately 0.1ml of a bacterial suspension (approximately 1 x 10^9 bacteria (Cfu/ml) was added to the test disinfectants (10ml), mixed thoroughly and left at room temperature for a specified contact time of 10 minutes. This experiment occupied quite a number of test tubes each, for the experiment was carried out in triplicates. Following contact an aliquot (1ml) was transferred to universal quenching agent, UQA (9ml of a solution containing 1g peptone,5g. Tween-80, 1g sodium thiosulphate and 0.7g lectithin 1^{-1} of deionised water pH7), for up to 60min to inactivate the disinfectants. The quenched solution were serially diluted in sterile distilled water and survivors enumerated on Nutrient Agar (Oxoid) using 0.1ml spread plates. The colonies on the plates were counted after incubation at 37^0C for 48hrs. Control test did not contain the disinfectants, but only the serially diluted suspension was plated and counted.

Preparation and Standardization of Salmonella Suspension

Salmonella species suspension was prepared from the stock culture through an overnight broth and standardized using 0.5 Mc Farland turbidity standard of standardization. Various concentrations of test disinfectants were prepared at 10% 20% 40% and 70%. Dettol, purit, savlon jIk, acetone, methanol, ethanol and phenol were prepared. Phenol co-efficient of these disinfectants was also calculated using Rideal Walker (1906),s method.

RESULTS

Figure 1.0 to 8.0 are graphical representations of disinfectant-resistant*Salmonella,* isolated from the study abattoirs at different concentrations.

Figure 1.0 showed the graph of disinfectants resistant *Salmonella* isolates. The number of colonies against study abattoirs at 10% concentration. In all the abattoirs, *Salmonella* species isolated was resistant, most, to Sodium hypochlorite (NaOCl) followed by purit, then savlon, lastly dettol. This shows that dettol was the best disinfectant to reduce *Salmonella* species to a minimal level at 10% concentration, followed by Savlon.

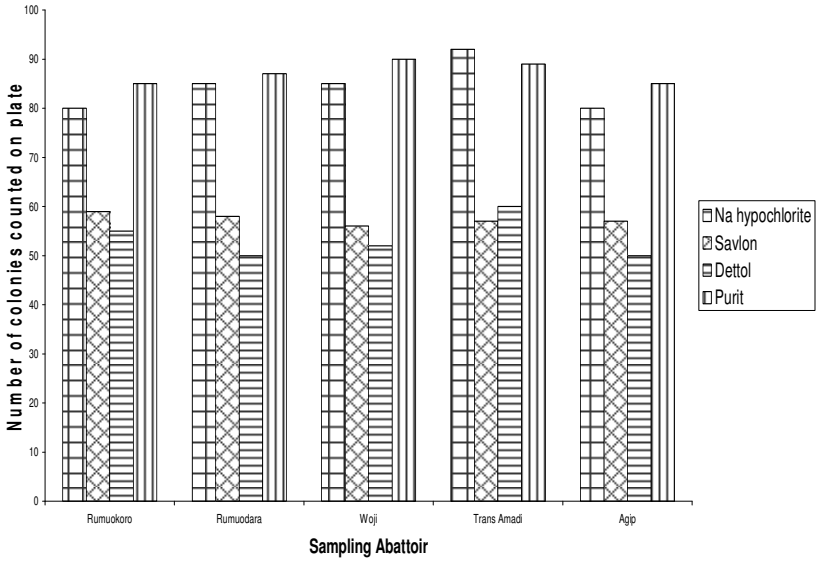

Figure 1.0: Disinfectants resistant *Salmonella* isolates at 10% concentration

Figure 2.0: showed the graph of Disinfectants resistant *Salmonella* isolates at 20% concentration. From the result of the graph, *Salmonella* was most sensitive to savlon at 20% concentration this means that the most potent disinfectant to *Salmonella* isolates at 20% concentration was savlon, followed by dettol, then purit and lastly Sodium hypochlorite.

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Figure 3.0: showed the graph of disinfectants resistant *Salmonella* isolates at 40% concentration. At this concentration, *Salmonella* isolates from the five different study abattoirs were more sensitive to dettol than to any other test disinfectants.

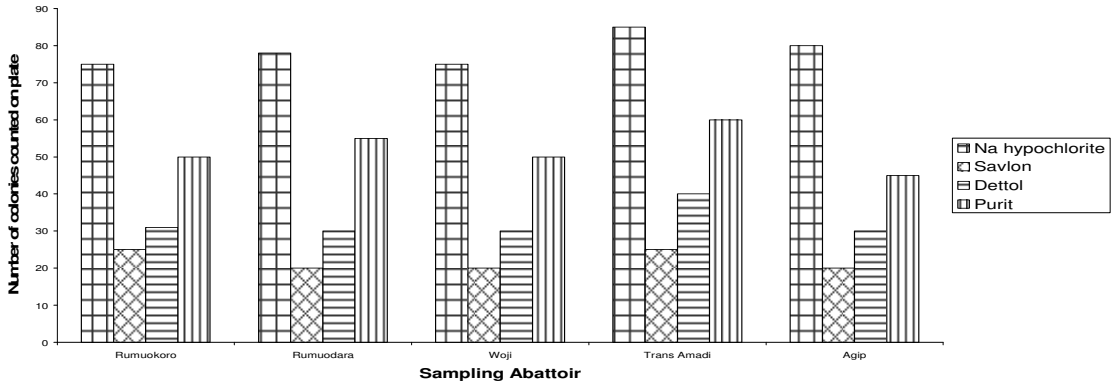

Figure 2.0: Disinfectants resistant *Salmonella* isolates at 20% concentration

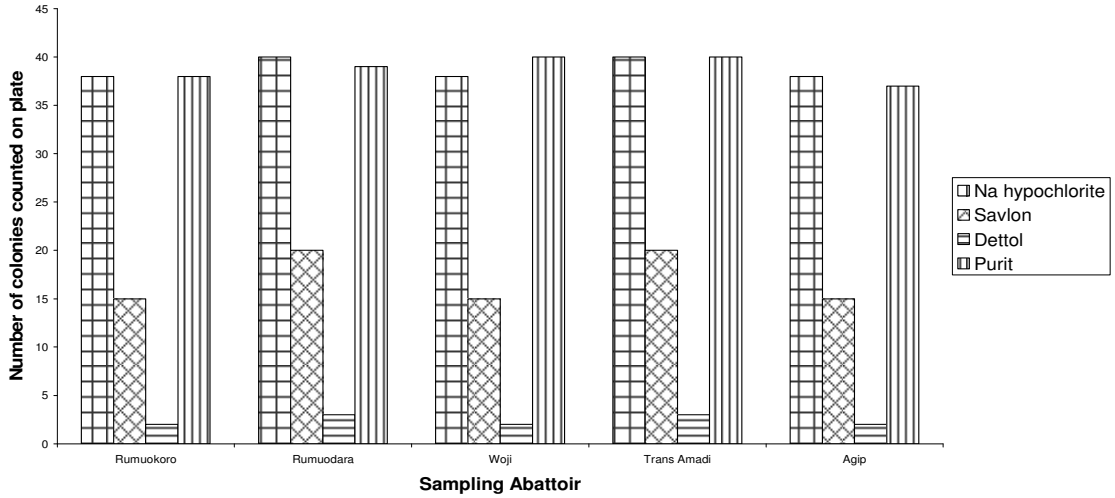

Figure 3.0: Disinfectants resistant *Salmonella* isolates at 40% concentration

Figure 4.0: showed the graph of disinfectants resistant *Salmonella* isolates at 70% concentration. From this graph, it could be deduced that dettol was highly resistant to *Salmonella* species isolated from these five study abattoirs at 70% concentration. Hence, dettol was the best disinfectant on these isolates at 70% concentration compared with others on these organisms.

ANOVA of all the microorganisms was calculated and tested at F - critical 0.05 level of significance.The result shows that there was a significant difference in the efficacy of all the disinfectants at different concentrations on microbial isolates.

Figure 5.0 shows the graphical representation of alcohol base disinfectants-disinfectant resistant *Salmonella* isolates at 10% concentration. *Salmonella* species were highly resistant to methanol followed by acetone, then ethanol and lastly, phenol. From this chart, it was observed that phenol was least resistant to *Salmonella*. Hence, this shows that *Salmonella* isolates were highly susceptible to phenol at 10% concentration.

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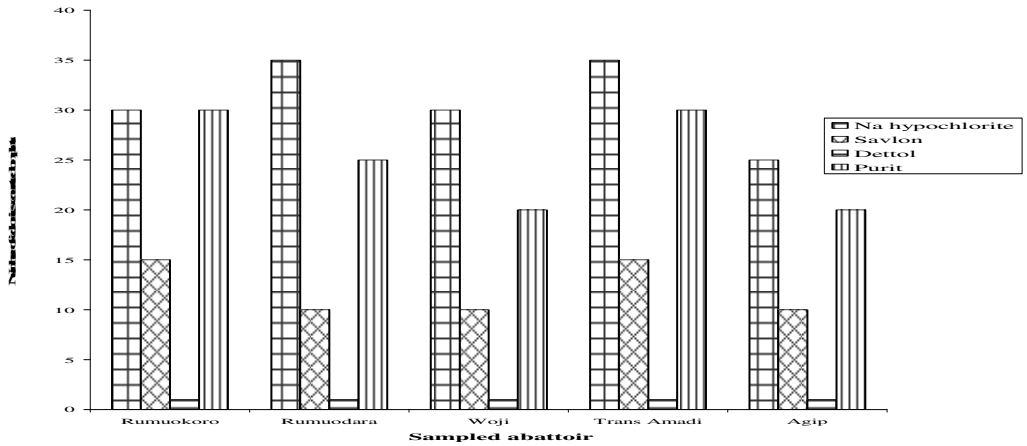

Figure 4.0: Disinfectants resistant *Salmonella* isolates at 70% concentration

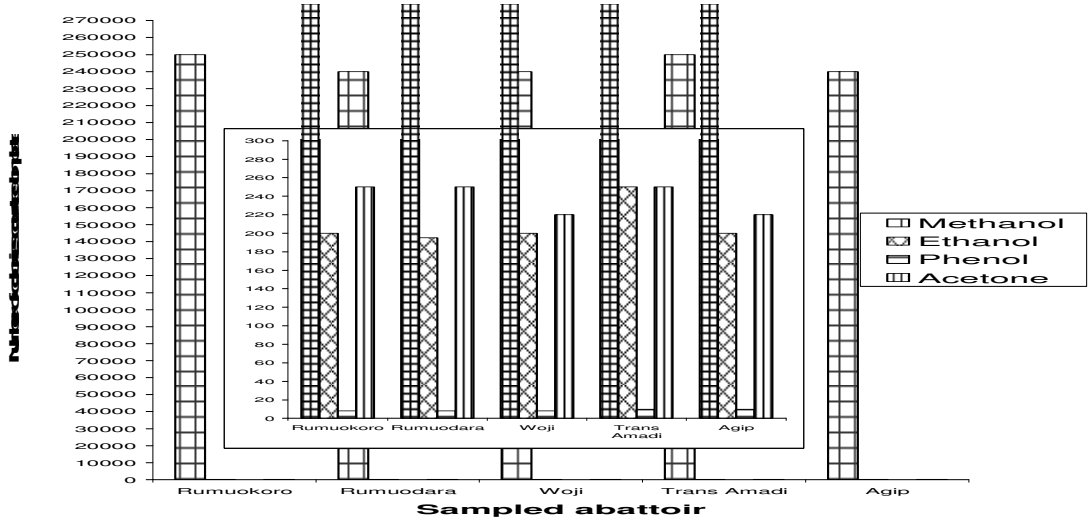

Figure 5.0: Alcohol base disinfectant-resistant *Salmonella* isolates at 10% concentration

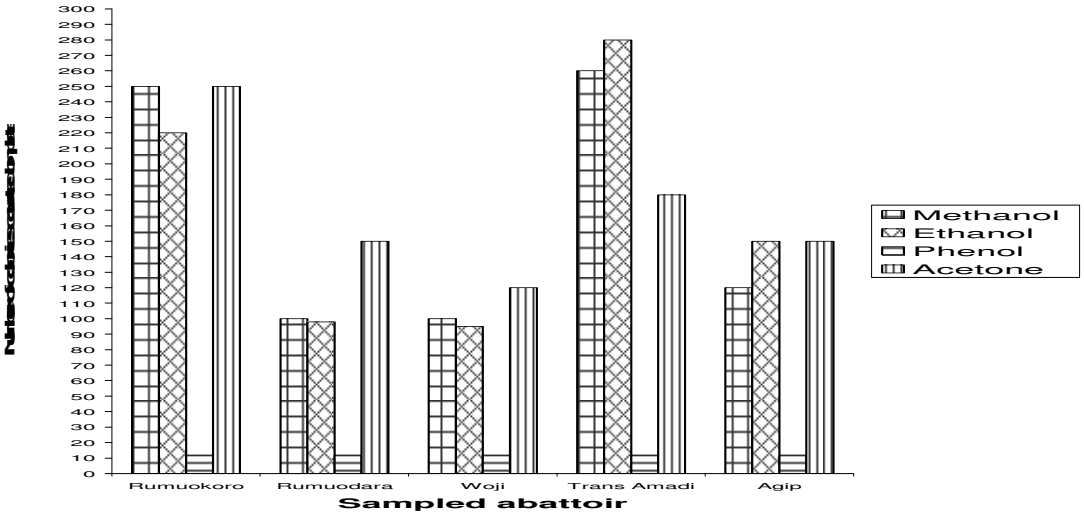

Figure 6.0: Alcohol base disinfectant-resistant *Salmonella* isolates at 20% concentration.

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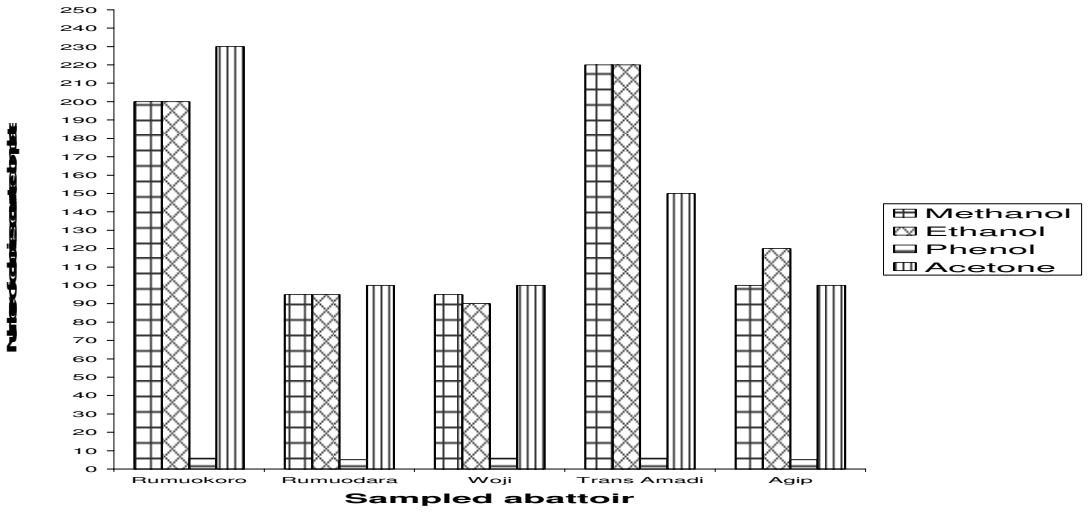

Figure 7.0: Alcohol base disinfectant-resistant *Salmonella* isolates at 40% concentration

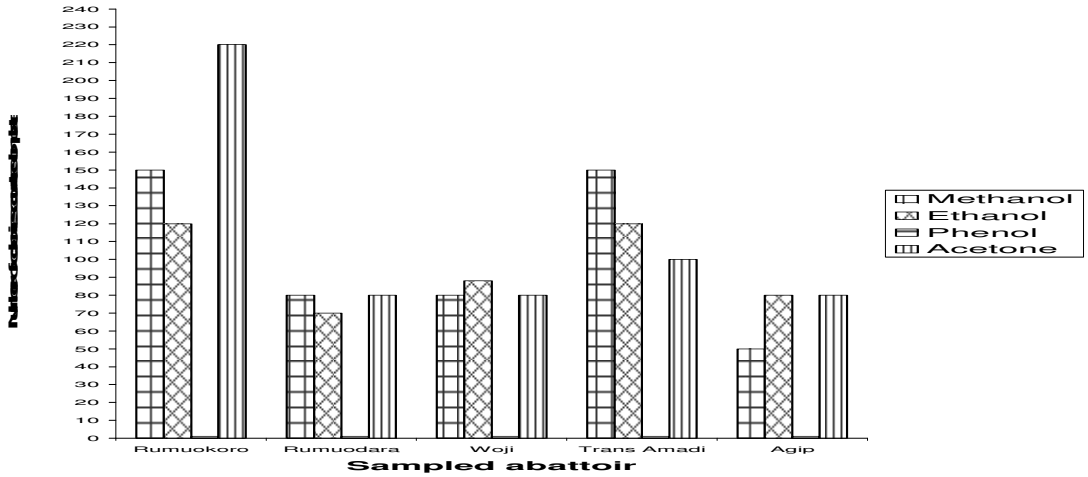

Figure 8.0: Alcohol base disinfectant-resistant *Salmonella* at 70% concentration

Figure 6.0 showed the graph of alcohol base disinfectants resistant *Salmonella* isolates at 20% concentration. From this chart, it can be deduced that phenol was most potent to *Salmonella* isolates.

Figure 7.0 shows the graph of alcohol base disinfectants resistant *Salmonella* at 40% concentration. The effectiveness of phenol was more than methanol, acetone and ethanol on *Salmonella* isolates from Rumuodara, Woji and Agip abattoirs than Rumuokoro and Trans Amadi abattoirs. This may be attributed to the different kinds of activities going on in these environment and their water bodies.

Figure 8.0 showed the chart of alcoholic derivatives resistant *Salmonella* isolates at 70% concentration. At this concentration, *Salmonella* species isolated in all these five study abattoirs were more sensitive to phenol disinfectants than to other alcohol base disinfectants. Only two colonies of *Salmonella* isolates were counted from the sensitivity plates after the exposure to phenol disinfectant at 70% concentration.

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DISCUSSION

This result revealed the most potent disinfectant among the test ones and this was dettol, followed by savlon. Purit and jik were not effective on *Salmonella* isolated from these abattoirs. These findings have shown that dettol can be used to sanitize the slaughter floor at 10% concentration , this will help reduce the microbial load of *Salmonella* species to a minimal and insignificant number of colonies. Four colonies were counted on the petri plates triplicates after the sanitizer was exposed to the test organism *Salmonella* species at 10% concentration. This number is insignificant and its ingestion from the meat cannot result into any disease or illness even in immunocompromised patients due to the acidic nature of the stomach. This work is in concurrent with the work of Ngo (2005) which stated that microflora was partly a reflection of the fish or meat processed and partly a reflection of the preservation parameters used in the products. The focus on safer foods and longer shelf-life has led to more frequent use of chemical disinfectants (Langsrud *et al*., 2003a). These findings indicate that there was no significant difference in the effectiveness of dettol, savlon and purit on *Salmonella* sp but there was a significant different between these disinfectants and Jik (Sodium hypochlorite). Jik potency on this organisms was poor, this is in concordance with the findings of Ngo (2005) which stated that the use of hypochlorite as a disinfectant in the presence of fat (herring juice) showed a week bactericidal efficiency. Though at 20% ,40% and 70% concentrations of exposing dettol and savlon to test organisms, the number of colonies counted were 2 to 0, which is an indication of increase in the potency of the test disinfectants, this is also in concordance with the findings of Holah *etal*., (2002) which stated that quaternary ammonium compound (QAC) have been used widely as disinfectants in seafood and food processing environment in developed countries such as the UK, and Norway, Bore and Langsrud (2005) stated that because of its low toxicity non-corrosiveness and high surface activity (Langsrud and Sundhein, 1997). However, several reports have described intrinsic and acquired resistance to these compounds especially among some Gram-negative species (Langsrud and Sundhein, 1997; Langsrud *et al*., 2003). Purit was not potent on these *Salmonella* sp isolated from slaughterhouse floor, this could be as a result of the presence of fats, which deactivated its efficacy, the reports by Norwood and Gilmour (1999) supported this view, and it stated that bacteria growth in this medium are more resistant to some disinfectants such as purit. The authors stated further that a reason why it is able to detect the bacteria survivors in this case might be due to the presence of fat. This might suggest that fat is the main factor affecting the bactericidal efficiency of the disinfectants, as the presence of fat might give the cells physical protection (Taylor *et al*.,1999). Acetone methanol, and ethanol were not good sanitisers, but phenol had a high potency on these isolates. Phenol would not be recommended due to its toxicity. The phenol coefficient calculated for the test disinfectants using Redial Walker method are dettol 7.0; purit, 1.7; savlon 2.0; jik 0.18; phenol 1.0; methanol 0.14; ethanol 0.14; and acetone 0.14.

Public Health Impact of Salmonella (Typhi) : Typhoid

Typhoid fever is caused by Salmonella typhi, a member of the genus Salmonella described earlier but distinguished by its inability to produce gas from carbohydrates; in many respect therefore, it is similar to the Shigellas. Typhoid is the most severe of the diseases caused by Salmonellas. After a lengthy incubation period of 7 to 21 days, the disease sets in with a general feeling of malaise and during the first week the body temperature steadily rises. About the seventh to tenth day, a rash appears and during the second week the fever is at its highest. Death may occur from the severity of the disease at this stage but in the less severe cases there is a gradual improvement in the third or fourth week. Salmonella typhi is excreted in the faeces in large numbers during the illness and as with salmonellosis, a carrier problem exists with patients who have recovered and with symptomless excreters. Sewage contaminated water is the most common source of infection but chlorination of water supplies has largely eliminated this source in developed countries.

The last big water-borne outbreak in the UK was in Croydon in 1937 when 431 people were infected by water that had been contaminated by a symptomless excreter repairing the mains system. Though two foods that used to be frequently incriminated in typhoid fever outbreaks were raw milk and ice-cream but the heat treatment regulations introduced in the United Kingdom in the 1940s overcame this source of infection. Raw milk was incriminated in an extensive outbreak in Bournemouth in 1938

when over 700 people were infected and 70 died. Actually ice-cream was the vehicle of infection in Aberystwyth in the same year when 210 cases were recorded. In both these examples the infection again stemmed from symptomless carriers and this stresses the problems that such people often unknowingly pose. Other foods associated with the disease have been shellfish including oysters, infected by the contaminated water in which they were living. Various canned meats have been responsible for a number of outbreaks in more recent years in the United Kingdom, the last major one being in Aberdeen in 1964 when 515 cases were reported. The original source of the organism was a South American tin of corned beef which had been cooled in contaminated water after cooking. *Salmonellatyphi* was undoubtedly spread to other meats in the shop by handling and by equipment; this outbreak was an excellent examples of the problems created by cross-contamination.

CONCLUSION AND RECOMMENDATIONS

The use of purit and Sodium hydrochlorite as disinfectants in the presence of fat in this study showed a weak bactericidal efficiency. Dettol and savlon were more effective than purit and hydrochlorite (Jik) on *Salmonella* sp isolated from 5 different abattoirs' (slaughterhouse) floor in Port Harcourt metropolis, Rivers State, Nigeria. From the result of this work, dettol was the most potent disinfectant on these test organisms, and is recommended for sanitizing the slaughter floor before placing animals for slaughter. The results also indicated that savlon could only be used in place of dettol if the latter is not available at that particular time of usage. Dettol has low toxicity and low corrosiveness, hence it is recommended at 10% concentration to sanitize this environment in order to deactivate the activities of these organisms in their present environment, thereby reduce the spread of typhoid fever in Port Harcourt city and in Nigeria as a whole.

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