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Disinfectant effects of Purit®, Z-Germicide® and Carcil® on bacterial isolates from hatcheries in Kaduna state, Nigeria

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Abstract
Hatcheries in Kaduna State, Nigeria were investigated for isolation of aerobic bacteria. The following were isolated: Escherichia coli, Proteus sp, Pseudomonas sp, Staphylococcus aureus, Staphylococcus sp and Micrococcus sp. On these isolates, the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and kill-time of two commonly used and one relatively new disinfectant were determined. The two commonly used disinfectants were Purit® (chlorhexidine gluconate Bp 0.3 % w/v and cetrimide Bp 3.0 % w/v) and Z-germicide® (7 % tar acid phenol and 2 % cresol). The new one was Carcil® (Alkyl-benzyl-dimethyl ammonium chloride). It was observed that Purit® was most effective against E.coli since it required the lowest concentration of 0.00026 % for 1 minute to kill the bacteria. Z-germicide® was found to be effective at 0.00063 % for 5 minutes and Carcil® required 0.0031 % for 10 seconds. Purit® was also found to be the most effective of the three disinfectants on Pseudomonas sp but Z-germicide® showed best results on Proteus sp.

Key words: Hatchery, bacteria, Purit, Z-germicide, Carcil, MIC, MBC and kill-time

Introduction

A chicken egg shell may have between 7,000 and 17,000 tiny pores out of which 1 % is open through which infectious agents can enter if the eggs are not handled properly (Fussell, 1987). Sources of the aforementioned agents could include: feedstuffs, wild birds, rodents, reptiles, man, pets, flies, soil, air, floors, drinkers, feeders, cages, faeces, egg trays and feathers (Bale *et al.*, 2002). Moulding and Wilson (1988) concluded that part of good sanitation practices often includes the treatment of eggs before and during incubation. Currently, most sanitation programmes require the use of some type of disinfectant. Control of microbial populations present on the shell and in the hatcheries requires a disinfectant that is effective in decreasing contamination but is not toxic to the developing embryos. Willinghan et al (1996) investigated bacterial resistance to hatchery disinfectants where three commercial chicken hatcheries were sampled for environmental bacteria. The bacteria isolated were tested for resistance to commercial preparations of quaternary ammonia, phenolic and glutaraldehyde liquid disinfectants. Approximately 8 % of the isolates from two of the three hatcheries were resistant to disinfectant concentrations at and above the manufacturers' recommended dilution and time of exposure. The resistant bacteria included Serratia marcescens, Bacillus cereus, Bacillus thuringiensis, Bacillus badius, Enterococcus fecalis, Enterococcus faecium, *Pseudomonas* stutzeri and Enterobacter agglomerans. This work was set to isolate aerobic bacteria which may be responsible for mortality of chicken embryos in the shells and to test the efficacy of the common disinfectants that were reported to be in use at the various hatcheries against bacteria. Some other common disinfectants were used in order to compare and also offer alternatives to the ones already in use.

Materials and Methods

Determination of MIC, MBC and the kill-time of disinfectants on the isolates

The disinfectants that were used in this study, were Z-germicide®, Purit® and Carcil®. These disinfectants were made up to working concentrations of 1 %, 0.03 % and 10 % respectively by diluting them in sterile distilled water. These working solutions were then subjected to a double-fold serial dilution in eleven test tubes each containing 3ml of double-strength nutrient broth. With these dilutions, the recommended concentrations by the manufacturers fell in the middle i.e. on the 6th test tube each. The double strength nutrient broth was to make room for the serial dilutions. Aliquots (0.1 ml) of 109 cfu/ml of bacterial suspension was added to each test tube and incubated at 37 0C for 24 hours after which the Minimum Inhibitory Concentration (MIC) was read as the concentration equivalent to the test tube that showed visibly complete clearance. The contents of 3 consecutive test tubes that showed clearance were plated on solid nutrient agar and incubated at 37 0C for 24 hours, then the 1st plate that showed no growth completely was taken as the Minimum Bactericidal Concentration (MBC).

To obtain the kill-time, a dilution of the disinfectant using normal saline was made to the MBC equivalent that was gotten. Aliquots (0.1 ml) of 109 CFU/ml of the bacterial suspension was inoculated into this concentration and then plating of the content using a loop-full at a time, was

done onto fresh nutrient agar plates at time intervals of 10 sec, 30 sec, 1 min, 2 min, 5 min and 10 min. This was done to determine the contact time of the disinfectant with the bacterium needed in order for the disinfectant to act on the bacterium by killing it. The plates were then incubated and results read after 24 and 48 hours at 37 0C. The first plate that yielded no growth completely showed the kill-time (Andrews, 2001).

Results

Table 1 showed the results for Z-germicide® indicating that E. coli required the least concentration of 63x10-5 (i.e. 1:1600) w/v of the disinfectant to kill the organisms and also at the least contact time of 5 minutes. *Proteus* and *Pseudomonas* on the other hand required higher concentrations of 12x10-4 (1:800) w/v for 10 minutes and 12x10-4 (1:800) w/v for more than 10 minutes respectively. In table 2, Purit® produced a similar result of 26x10-5 (1:3840) w/v for 1 minute on E. coli, 21x10-4 (1:480) w/v for 5 minutes on *Proteus* and 42x10-4 (1:240) w/v for 5 minutes on *Pseudomonas*. Table 3 showed that Carcil® was effective at 31x10-4 (1:320) w/v for 10 seconds on E. coli, 31x10-4 (1:3200) w/v for 30 seconds on *Proteus* and 62x10-4 (1:160) w/v for 10 seconds on *Pseudomonas*.

Table 1: Minimum inhibitory concentration, minimum bacterial concentration and kill-time of Z-germicide® on hatchery bacterial isolates

Isolate	MIC (W/v)	MBC (W/v)	Kill-time (seconds)
Escherichia coli	16×10^{-5}	63×10^{-5}	300
Proteus sp	31×10^{-5}	12×10^{-4}	600
Pseudomonas sp	31×10^{-5}	12×10^{-4}	> 600

Table 2: Minimum inhibitory concentration, minimum bacterial concentration and kill-time of Purit® on hatchery bacterial isolates

Isolate	MIC (W/v)	MBC (W/v)	Kill-time (seconds)
Escherichia coli	$65x10^{-6}$	$26x10^{-5}$	60
Proteus sp	$52x10^{-5}$	$21x10^{-4}$	300
Pseudomonas sp	$10x10^{-4}$	$42x10^{-4}$	300

Table 3: Minimum inhibitory concentration, minimum bacterial concentration and kill-time of Carcil ® on hatchery bacterial isolates

Isolate	MIC (W/v)	MBC (W/v)	Kill-time (seconds)
Escherichia coli	$78x10^{-5}$	$31x10^{-4}$	10
Proteus sp	$78x10^{-5}$	$31x10^{-4}$	30
Pseudomonas sp	$16x10^{-4}$	$62x10^{-4}$	10

Discussion

The effects of disinfectants were determined only on the Gram -ve bacteria because works by Mamman et al (2005) showed that Gram -ve bacteria were generally more resistant to effects by disinfectants than Gram +ve bacteria probably due to their having a more complex cell wall. Therefore, the results from this work will not hold a bearing on the Gram +ve bacteria. The three disinfectants used (Carcil®, Purit® and Z-germicide®) were found to be effective against the bacterial isolates but at different concentrations and contact time (Tables 1, 2 and 3).

Purit® was found to be most effective against E. coli since it required the lowest concentration of 0.00026% and 1 minute contact time to kill the bacteria. This was followed by Z-germicide® requiring 0.00063% for 5 minutes then Carcil® requiring 0.0031% for 10 seconds. On *Proteus*, Z-germicide® was most effective requiring 0.0012% for 10 minutes followed by Purit® at 0.0021% for 5 minutes and then Carcil® at 0.0031% for 6 seconds. In the same vein, Z-germicide® acting on *Pseudomonas* was found most effective since it acted at 0.0012% but for greater than 10 minutes and followed again by Purit® at 0.0042% for 5 minutes then Carcil® at 0.0062% for 10 seconds. These results contrast with those of Mamman et al (2005) where Carcil® was seen to kill E. coli isolates from clinical cases handled at the Ahmadu Bello University Veterinary Teaching Hospital where the effect was seen at 0.039% for 1 minute but on *Proteus*, concentration had to be raised to 0.625% for 20 seconds. The most important pathogenic bacteria recovered in this study were E. coli and Staphylococcus aureus. Purit® (chlorhexidine gluconate and cetrimide) has proved to be the most effective of the three disinfectants used. It is therefore safe to recommend this to the hatcheries for use. It has been reported that chlorhexidine gluconate had been recommended as a water pan additive in incubators and borders because it has been found to be effective against Aspergillus fungus and Newcastle disease virus; it is not corrosive to equipments, readily available and with moderate cost (Johnson, 1996). But its disadvantages include poor efficiency against other viruses and Gram -ve bacteria especially *Pseudomonas*, ineffectiveness in the presence of organic debris and against bacterial spores, it must be discarded and remixed daily (Johnson, 1996). In efficiency, Purit® was closely followed by Z-germicide® which has phenol and cresol as its active ingredients.

The most common disinfectant reported to be used in the various hatcheries investigated was Morigad® which has phenol as its active ingredient. It is thus reasonable to say that the hatcheries could continue to use this disinfectant but at a little higher concentration than recommended by the manufacturers. This recommendation is because Morigad® shares the same active ingredient with Z-germicide® which is phenol and since the efficacy of Z-germicide® was demonstrated in this study Morigad® could be used. The recovery of microbes from the same eggs that were treated with Morigad® suggested that the hatcheries were either using lower doses or the manufacturers' recommended dose was slightly low;

therefore an increased concentration should be used. Since hatcheries deal with eggs under incubation (a process which takes a long time to complete), it is therefore imperative to acquire disinfectants that have a long and safe residual effect. The incorporation of cresol in Z-germicide® which is known to have long residual effect and also reported to be effective even in the presence of organic matter makes Z-germicide® a superior phenolic disinfectant than the morigad® being used currently. The major disadvantage of cresol is the release of noxious gases.

In view of the disadvantages associated with the aforementioned disinfectants, an alternative was sought which was Carcil® a quaternary ammonium compound. The quaternary ammonium compounds may be the most commonly used disinfectants for equipments like incubators and hatching trays because they are relatively non-irritating, non-corrosive, of low toxicity and reasonably effective in the presence of organic matter. Since the incubator and its components should be cleared free of organic matter before applying a disinfectant, quaternary ammonium compounds make a good choice (Haynes and Smith, 2003).

Conclusion and recommendations

Results obtained in this study showed that the disinfectants were effective at higher concentrations than the recommended levels by the manufacturers; this may be due partly to some level of resistance that might have developed by the microbes against these disinfectants. The hatchery managers are therefore advised to raise the concentrations of the solutions used for disinfection to the MBC levels recorded. It is recommended that Morigad@ which in this study was found to be mostly used by all the hatcheries should be continued but at a higher concentration with care. Three other disinfectants as used in this study are also recommended for use at alternate intervals to curb resistance. Finally, appropriate hatchery sanitation is recommended which involves setting up of clean eggs in the incubators, hatchery personnels to maintain a high level of personal hygiene during hatchery procedures, also employing the use of clean incubators, etc. these, if observed will reduce dead-in-shell embryos due to bacteria and in turn may improve poultry productivity.

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