BACTERIAL FLORA OF HATCHERY ENVIRONMENT AND THEIR *IN-VITRO* SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

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ABSTRACT

Four hatcheries, located in and around Faisalabad, were sampled a day before hatch out in six batches for environmental bacterial flora. Hatchery air, egg-shell surface, surfaces of selected locations and water supply samples were taken for this purpose. The percent (relative) occurrence of various bacterial species recovered from hatchery environment revealed that *Bacillus subtilis* was the predominant isolate (26.93%), followed by *Escherichia coli* (24.08%), *Staphylococcus epidermidis* (16.32%), *Staphylococcus aureus* (8.16%), *Paratyphoid salmonellae* (6.93%), *Pseudomonas aeruginosa* (4.48%), *Citrobacter freundii* (4.08%), *Enterococcus faecalis* (3.26%), *Klebsiella pneumoniae* (3.26%), *Bordetella avium* (1.63%) and *Proteus vulgaris* (0.81%). In second part of the study, bacterial isolates were subjected to *in-vitro* antibiotic sensitivity to 8 antibiotics of common poultry use. It was found that 98.92, 79.56, 65.59, 61.29, 61.29, 61.29, 53.76 and 38.70 percent of bacterial isolates were sensitive to Norfloxacin, Gentamicin, Neomycin, Chloramphenicol, Doxycycline, Flumequine, Erythromycin, and Ampicillin, respectively. In the final part of the study, bacterial isolates were tested for resistance to 3 commerical hatchery disinfectants (TH_4 [®], Aldekol Des[®] 0.2, and Bromosept 10% soln. [®]). Only 3.22% of the isolates showed resistance at manufacturer's recommended dilution (MRD) levels, while 11.82% of the isolates showed resistance at concentrations below the MRD levels.

Key words: Bacterial flora, susceptibility, hatcheries.

INTRODUCTION

Efficiency of hatchery sanitation procedure is evaluated by microbiological examination of fluff, dead-in-shell (DIS) embryos and hatchery environment. Examination of fluff and DIS embryos indicates the presence of pathogenic microorganisms in the hatchery environment. However, the probe into the sources and reservoirs of these contaminants within hatchery and microbiological examination of hatchery environment are required. Application of effective disinfectant at manufacturer's recommended dilution level is included in any hatchery sanitation program (Deeming, 1998). If these recommendations are not adhered to, the potential for selection of resistant population of bacteria may get exacerbate (Willinghan *et al*., 1996). As bacterial sensitivity profile to antibiotics of common poultry use changes from time to time, there is need to update our knowledge of current profile of bacterial susceptibility to these antibiotics. Considering these facts the present project was planned with the following objectives:

- To determine the environmental bacterial flora at different hatchery locations and to study the potential effect of its presence on hatchability and day-of-hatch chick viability.
- To determine the *in-vitro* susceptibility profile of bacterial isolates to antibiotics and hatchery disinfectants.

MATERIALS AND METHODS

Four hatcheries (A, B, C and D) were sampled a day before hatch out in six batches. Hatcheries A and B were sampled twice while hatcheries C and D were sampled once. Hatchery air, egg-shell surface and surfaces of selected locations within hatchery were sampled according to Williams *et al*. (1980), while water supply was sampled according to the method described by Senior (1989). Environmental samples were cultured and bacterial colonies were isolated and following purification were identified according to the schemes outlined by Krieg and Holt (1984). For *Staph. aureus, Staph. epidermidis, Bacillus subtilis, E. coli and Paratyphoid (PT) salmonellae,* 10 isolates were randomly selected from a lot of each species and were subjected to sensitivity testing against 8 antibiotics and 3 commercial hatchery disinfectants. For *Enterococcus faecalis, Klebsiella pneumoniae, Citrobacter freundii, Pseudomonas aeruginosa, Bordetella avium* and *Proteus vulgaris* all the isolates were tested.

In second part of the study, the bacterial isolates were subjected to *in-vitro* sensitivity testing. Disc diffusion technique was used according to the method of Kirby and Bauer, as described by Scott (1989). The results were recorded by considering the clear zone around different antibiotic discs, as sensitive or no clear zone around the discs as resistant. In final part of the study, bacterial isolates were tested for resistance to 3 commercial hatchery disinfectants (TH_4®, Aldekol Des® 0.2, Bromosept 10% solution) following the method described by Willinghan *et al.* (1996). These disinfectant preparations were mixed with sterilized, distilled, deionized water at dilutions below and above the manufacturer's recommended dilutions. Both the Aldekol and Bromosept 10% were used at dilutions of 1:200, 1:250, 1:300, 1:350, 1:400, 1:450, 1:500, 1:550 and 1:600. The manufacturer's recommendations call for a 1:400 dilution. TH_4 was used at dilutions of 1:800, 1:850, 1:900,1:950, 1:1000, 1:1050, 1:1100, 1:150 and 1:1200. The manufacturers' recommendations call for a 1:1000 dilution. In this case the results were recorded as resistant (growth) or sensitive (no growth). The growth was compared with the respective control broth (no bacteria was added).

RESULTS AND DISCUSSION

It is widely accepted that bacterial contamination is a contributory factor towards reduced hatchability. Bacteria can affect the hatchability of embryo once inside the egg. However, the type of bacterium is important and some are more likely to reduce hatchability than others. Eggs are exposed to non-pathogenic bacteria resulting in either the subsequent death of embryo or lower the chick viability. The hygienic status of the environment into which an egg is incubated is thought to play an important part in increasing the likelihood of bacterial penetration (Bruce and Drysdale, 1991). In this study, a total of 245 bacterial isolates were identified. The percent (relative) occurrence of various bacterial species recovered from hatchery environment is presented in Table 1. This shows that majority of the isolates are Gram positive (54.67%). Table-2 shows percent (relative) occurrence of bacterial species recovered through environmental sampling within particular hatchery locations. The term incubation room was reserved for hatchery B where both the hatcher and setter are installed in the same room. From water supply samples, 2.85% of total isolates (245) were recovered. The percent (relative) occurrence of different bacterial species isolated from water supply samples showed that 5 (71.4%) isolates were *E.coli*, (4.28%) were *Ps. aeruginosa* and 1 (14.28%) were salmonellae. These organisms become the part of hatchery environment from various sources. Air flow, employee activity, soiled egg shells and contaminated water supply are responsible for the dissemination of these contaminants within hatchery environment. Anyhow, whatever the source might be, the ultimate destination of these contaminants was setter and hatcher where they manifested the effect of their presence. When these contaminants are present within setter at a reasonably high level then their presence is manifested in the form of increased number of dead-in-shell embryos (Sarakbi 1989; Abd-El-Galil *et al.*, 1995; Khan, 1997). Venkanagouda and Upadhye (1996) pointed out the assoication of organisms, recovered form hatcher environment, with omphalitis and early chick mortality. According to Barnes (1997), *Bord. avium* alters the host susceptibility to *E. coli* infection in poultry while the presence of *K. pneumoniae* increases the severity of respiratory diseases resulting from *Bord. avium*. In addition, some of these organisms have role in the ecosystem of hatcher. According to Baba *et al.* (1991), *E. coli* might play a role of competitive exclusion (CE)

Table 1. Percent (relative) occurrence of different bacterial species isolated through environmental sampling

Bacterial species	No. of isolates	Percent
Staphylococcus aureus	20	8.16
Staphylococcus epidermidis	40	16.32
Bacillus subtilis	66	26.93
Escherichia coli	59	24.08
Enterococcus faecalis	8	3.26
Klebsiella pneumoniae	8	3.26
Citrobacter freundii	10	4.08
Pseudomonas aeruginosa	11	4.48
Bordetella avium	4	1.63
Paratyphoid salmonellae	17	6.93
Proteus vulgaris	2	0.81

in newly hatched chicks, in inhibiting the colonization of *Sal. typhimurium* in the caeca. Likewise Staphylococcus species are considered to be normal gut flora, which help suppress other possible pathogens by their presence through CE (Skeeles, 1997). Devriese *et al.* (1991) reported that *Ent. faecalis* largely dominated the enterococcal and streptococcal gut flora of 1-day-old chicks. The importance of this early colonization by *Ent. faecalis* lies in the fact that this organism inhibits the growth of *Sal. typhimurium* and *E.coli in-vitro* (Hinton *et al.*, 1992). Out of all the species recovered from hatchery environment salmonellae was regarded by Khan (1997) as indicator organism for the poor managemental and unhygienic conditions prevailing at our hatcheries. It also has a zoonotic importance and attendants may act as a source of these organisms within hatchery (Symth and Watson, 1987).

The *in-vitro* antibiotic sensitivity testing of the bacterial isolates to 8 antibiotics of common poultry use showed that 98.92, 79.56, 65.59, 61.29, 61.29, 61.29, 53.76 and 38.70 percent of bacterial isolates were sensitive to Norfloxacin, Gentamicin, Neomycin, Chloramphenicol, Doxycycline, Flumequine, Erythromycin, and Ampicillin, respectively. Multiple antibioitc resistance as indicated by Khan (1997) was also a main feature of this study. Another important feature was resistance (1.08%) of isolates to Norfloxacin. This resistance was already reported by Prasad *et al.* (1997) and Saleem (1998). As transfer of resistance is a common feature among enterobacteriaceae (Gast and Stephens, 1986), it is professed that in future, because of indiscriminate use of quinolones in poultry industry, the present drugs of this group would no longer remain the last resort.

Sensitivity of the bacterial isolates was tested to 3 commercial hatchery disinfectants (TH_4^+, Aldekol Des 0.2, and Bromosept 10%). Only 3.22 percent of the isolates tested showed resistance at manufacturers' recommended dilution (MRD) levels, while 11.82 percent of the isolates showed resistance at concentrations below the MRD levels. Overall percent efficacy of Aldekol, Bromosept 10% and TH_4^+ was 94.62, 95.69 and 94.62 percent, respectively. For Aldekol, 5.37 percent of the isolates showed resistance. Out of these resistant isolates, 80 percent showed low level of resistance and 20 percent showed moderate resistance. For Bromosept 10%, 4.3 percent of isolates showed resistance. Out of these resistant isolates, 50% showed low level of resistance and another 50% showed moderate resistance. For TH_4^+, 5.37% of isolates showed low level of resistance. For Aldekol, Bromosept 10% and TH_4^+, 10.07, 2.15 and 0 percent of the isolates showed moderate level of resistance, while 4.30, 2.15 and 5.37 percent of isolates showed low level of resistance, respectively.

In-vitro antimicrobial susceptibility profile of bacterial flora of hatchery environment to Aldekol, Bromosept 10% and TH_4^+ is presented in Table 3. Ten percent of isolates of *Staph aureus* showed low level of resistance to Aldekol. According to Gardner and Phil (1977), Staphylococci are usually highly susceptible to biocides intrinsically. Acquired resistance does occur in *Staph. aureus* to some biocides (Russel, 1998). According to Willinghan *et al.* (1996), resistance in case of *B. subtilis* is not so important as it is a non-pathogenic organism for chick embryo and day-of-hatch chicks. *Ent. faecalis* showed moderate level of resistance to Bromosept 10% and low level of resistance to TH_4^+. Willinghan *et al.* (1996) also reported the resistance of this organism ranging from high to low levels. In the present study *Ps. aeruginosa* also showed varying levels of resistance to these three disinfectants. According to Russell (1998), Pseudomonas is intrinsically less sensitive to a variety of chemically unrelated biocides. Acquired resistance may occur as a results of mutation or by the acquisition of plasmids or transposons. In addition to these resistance properties, Pseudomonas is able to degrade quaternary ammonium compounds (Raymond and Alexander, 1977).

Table 2. Percent (relative) occurrence of bacterial species recovered through environmental sampling within particular hatchery locations

Bacterial species	Hatcher room	Setter room	Hatcher + eggs in it	Setter + eggs in it	Attendants' room	Incubation room
Staph.aureus	5.55	5.88	8.95	6.25	27.27	15.38
Staph.epidermidis	5.55	17.64	17.91	17.85	27.27	7.69
B. subtilis	38.88	41.17	26.86	25.00	18.18	30.76
E. coli	33.33	23.52	20.89	23.21	18.18	15.38
Ent. faecalis	--	5.88	5.97	1.78	--	7.69
K. pneumoniae	--	--	1.49	5.35	--	7.69
C. freundii	--	--	1.49	8.03	--	--
Ps. aeruginosa	16.66	--	2.98	4.46	--	--
Bord. avium	--	--	5.97	--	--	--
Parathyphoid salmonelae	--	5.88	5.97	7.14	9.09	15.38
P. vulgaris	--	--	1.49	0.89	--	--

Table 3: In-vitro antimicrobial susceptibility profile (%) of bacterial flora of hatchery environment to three commercial hatchery disinfectants

Bacterial species	Aldekol Des ® 0.2				Bromosept 10% Soln. ®				TH_4^+®			
	Sensitive	Resistant			Sensitive	Resistant			Sensitive	Resistant		
		H	M	L		H	M	L		H	M	L
Staph. Aureus	90	--	--	10	100	--	--	--	100	--	--	--
Staph. epidermidis	100	--	--		100	--	--	--	100	--	--	--
B.subtilis	90	--	--	10	90	--	--	10	80	--	--	20
E.coli	100	--	--	--	100	--		--	100	--	--	
Ent. faecalis	100	--	--	--	87.5	--	12.5	--	87.5	--	--	12.5
K. pneumoniae	100	--	--	--	100	--		--	100	--	--	--
C.freundii	100	--	--	--	100	--		--	100	--	--	--
Ps. aeruginosa	63.63	--	--	37.37	81.81	--	9.09	9.09	81.81	--	--	18.18
Bord. avium	100	--	--	--	100	--	--	--	100	--	--	--
Parathyphoid salmonelae	100	--	--	--	100	--	--	--	100	--	--	--
P. vulgaris	100	--	--	--	100	--	--	--	100	--	--	--

H = High level resistance M = Moderate resistance L = Low level resistance

Although *in-vitro* bacterial resistance to three commercial disinfectants tested was very low yet it is anticipated that in actual hatchery conditions, a higher number of bacteria would show resistance to these three disinfectants. So there is need to check the efficacy of these chemicals at hatchery level to have an exact profile of bacterial resistance to these disinfectants. It is worthwhile to mention that this study was not intended to relate actual laboratory resistance to field levels of disinfectants. So it is beyond the scope of this *in-vitro* method to cast a verdict about the efficacy of these 3 commercial preparations in actual hatchery conditions. At the same time on the basis of these results it is impossible to make any comparison between these three disinfectants.

REFERENCES

Abd-El-Galil, Y., A.I. El-Kenawy, S.R. Gmiey, and M.M. Abd-El-Latif, 1995. Bacterial causes of lowering hatchability and early embryonic chicken deaths in Balady hatcheries in Dakahila Governorate. Assuit Vet. Med. J., 33: 199-206.

Baba, E., S. Nagaiashi, T. Fukata and A. Arakawa, 1991. The role of intestinal microflora on the prevention of Salmonella colonization in gnotobiotic chickens. Poult. Sci., 70(9): 1902-1907.

Barnes, H.J., 1997. Other bacterial diseases. In: Diseases of Poultry, B.W. Calnek, H.J., Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (eds) 10th ed., Iowa State University Press Ames, Iowa, USA. 289-296.

Bruce, J. and E.M. Drysdale, 1991. Egg hygiene: routes of infection. In: Avian Incubation, S.G. Tullet (ed.). Buttersworth and Co. (Publishers) Ltd., UK: 257-267.

Deeming, D.C., 1998. Vulnerability of the embryo to hatching egg disinfection. World Poult., 14(9): 29-30.

Devriese, L.A., J. Hommez, R. Wijfels and F. Haesebrouck, 1991. Composition of the enterococcal and streptococcal intestinal flora of poultry. J. Appl. Bact., 46-50.

Gardner, J.F. and D. 1977. Principles of antimicrobial activity Disinfecton, Sterlization and Preservation, S ck (editor) 2nd ed. Lea and Febiger Philad 883-907.

Gast, R.K. and J.f. Ste 1986. *In-vitro* transfer of antibiotic resista strains of *Salmonella arizonae*. Poult. 70-279.

Hinton, A., D.E. Cu hatcher J.R. Deloach, 1992. *In-vitro* inhibition gemental *nella typhimurium* and *Escherichia c* nimal Scien by an anaerobic Gram-positive cocc and water om the cecal contents of adult chicke tec., 55(3): 162-166.

Khan, M.A., 1997. Ba of dead-in-shell broiler embryos and antibiotic sensitivity of the isolates. M.Sc.(Hons.) Thesis, Dept. Vet. Microbiol., Univ. Agri. Faisalabad.

Krieg, N.R. and J.G. Holt, 1984. Bergey's Manual of Systemic Bacteriology, 9th ed., Williams and Wilkins, Baltimore/London.

Prasad, V., K.K. Murthy and T.V.J. Rao, 1997. *In-vitro* antibiogram studies of *Escherichia coli* in chickens. Ind. Vet. J., 74: 616-617.

Raymond, D.D. and M. Alexander, 1977. Bacterial metabolism of quaternary ammonium compounds. Appl. Envir. Microbiol., 33(5): 1037-1041.

Russell, A.D., 1998. Microbial susceptibility and resistance to chemical and physical agents. In: Topley and Wilson's Microbiology and Microbial Infections, Vol. II, Systemic Bacteriology, A. Balows and B.I. Duerden, (eds.) 9th ed., Hodder Headline Group, London, : 149-184.

Saleem, M., 1998. Avian *Escherichia coli* infection (I) *In-vitro* antibiotic susceptibility profiles and (II) *In-vitro* efficacy of enrofloxacin in induced diseases. M.Sc. Thesis, Deptt. C.M.S., Univ. Agri. Faisalabad.

Sarakbi, T., 1989. Klebsiella-a killer in the hatchery. Intl. Hatch. Prac., 3(5): 19-21.

Scott, A.C., 1989. Laboratory control of antimicrobial therapy. In: Mackie and McCartney Practical Medical Microbiology, J.G. Collee, J.P. Dugid, A.G. Fraser and B.P. Marmion (eds.) Vol. II, 13th ed., Churchill Livingstone Edinburgh, London: 161-171.

Senior, B.W., 1989. Collection of water samples. In: Mackie and McCartney Practical Medical Microbiology, J.G. Collee, J.P. Dugid, A.G. Fraser and B.P. Marmion (eds.), Vol. II, 13th ed., Churchill Livingstone Edinburgh, London, ; 204.

Skeeles, J.K., 1997. Staphylococcus. In: Diseases of Poultry, B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (eds.) 10th ed., Iowa State University Press Ames, Iowa, USA: 247-253.

Smyth, F.B. and J.D. Watson, 1987. Salmonella in a chicken hatchery. Ulster Med. J. 56(2): 157-159.

Venkanagouda, G.K. and A.S. Upadhye, 1996. Bacterial etiology of early chick mortality. Ind. Vet. J., 73: 253-256.

Williams, J.E., E.J. Mallinson and G.H. Snoeyenbos, 1980. Salmonellosis and Arizonosis. In: Isolation and Identification of Avian Pathogens, S.B. Hitchner, C.H. Domermuth, H.G. Purchase, J. E. Williams (eds.). The American Association of Avian Pathologists. : 2-4.

Willinghan, E.M., J.E. Sander, S.G. Thayer and J.L. Wilson, 1996. Investigation of bacterial resistance to hatchery disinfectants. Avian Dis., 40(3): 510-515.