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Pakistan Journal of Biological Sciences, 2 (2): 442-444, 1999 **Research Article**

Bacteriology of Dead-in-Shell Broiler Embryos and Antibiotic Sensitivity of the Isolates

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Abstract

A total of 105 dead-in-shell embryos, randomly selected from three local hatcheries of Faisalabad were bacteriologic examined. The occurrence of dead-in-shell was recorded to be 9.91 per cent (8.04 to 12.03%) positive liver samples dead-in-shell embryos. The relative occurrence of different bacterial species were: Escherichia coli 52.54 per cen *Paratyphoid salmonellae* 12.7 per cent, *S. Gallinarum* 11.86 per cent. *S. Pullorum* 5.93 per cent, *Streptococcus faeca* 5.93 per cent, *Bacillus subtilis* 4.2 per cent, *Pseudomonas aeruginosa* 3.39 per cent and *Proteus mirabilis* 3.39 per cen 28.26 per cent positive samples yield mixed growth of two different bacterial species and *E. coli* was found in all of the combinations. 91.94 per cent of the *E. coli* isolates were found Congo red positive. The in vitro sensitivity of the isolat to the 11 antibiotics were as: norfloxacin 100 per cent, gentamicin 89 per cent, flumequine 80 per cent, neomycin 68.6 per cent, chloramphenicol 67.80 per cent, streptopenicillin 65 per cent, erythromycin 14.4 per cent and furazolidone 13. per cent. Multiple drug resistance was observed among the isolates, particularly against the antibiotics frequently used poultry.

Introduction

For effective management and disease control, poultry production can be divided into the stages of incubation and hatching, brooding, rearing and adult or laying. All of these stages are affected by diseases and need equal attention for a profitable poultry production (Nesheim *et al.*, 1979).

Hatchery is one of the greatest areas of disease risk in the whole cycle of poultry operations. A large proportion of embryos die at different stages of incubation (Jordan, 1990). Bacterial and fungal contamination of the hatching eggs is important as they not only prevent more than 10 per cent of fertile eggs from hatching but may also give rise to cross contamination and subsequent mortality or poor performance of chicks in the subsequent stages of production (Anonymou, 1996). Bacterial causes of mortality in chicken during the stages of brooding, rearing and laying were investigated in Pakistan. The present reports the bacterial causes of dead-in-shell during hatchery.

Materials and Methods

Collection of samples: A total of 105 dead-in-shells broiler embryos were procured from three different local hatcheries (in seven batches) in and around Faisalabad. The occurrence of dead-in-shells and unhatched eggs was recorded from the hatchery record.

Bacterial isolation: Liver samples from the embryos were inoculated aseptically on nutrient agar blood agar and Mackoukey's agar. The inoculated plates were incubated aerobically at 37°C. The bacteria isolated were subjected to bacteriological identification. The isolates were characterized on the basis of cultural, morphological characteristics, sugar fermentation and biochemical reactions (Krieg and Holt, 1984; Collee *et al.*, 1989). Pathogenicity test for Escherichia coli and Congo red (CR binding test were performed to detect the pathogenicity the isolated E. Coli strains.

***In vitro* antibiotic sensitivity:** All the bacterial isolates we tested *in vitro* for their sensitivity to 11 different antibiotic of veterinary importance. Standard disc diffusion techniqu described by Scott (1989) was used. The results we recorded the clear zones around different antibiotic disk as sensitive or no clear zones around the disks as resistan

Results and Discussion

Bacteria may contaminate the content of hen eggs as the form in the oviduct but contamination after laying is mo likely (Anonymou, 1996). Embryonic mortality at the la stage (16-21 days) of incubation accounts for more than 6 per cent of the total embryonic mortality and is mo closely related to the bacterial infection (Jordan, 1990). Th occurrence of dead-in-shells 9.91 per cent (8.04 t 12.09%) of the total eggs set is not much higher than th 9 per cent, declared in the surveys on broiler breeder eg in U.K. but the occurrence of unhatched eggs 21.23 p cent (16-26.66% Table 1) is higher than the 17 per cent found in the above surveys in U.K. (Hordan, 1990).

Bacterial contamination was attributed by 87.62 per cent the examined dead-in-shell which is significantly higher th the 45 per cent bacterial infections of dead-in-shell (Tuch *et al.*, 1996).

A total of 118 bacterial species were isolated. The relati occurrence of these bacterial species is shown in Table It indicates that majority of the isolates are gram negati coliforms. *E. coli* is the main isolate 52.54 per cent of th

able 1: Occurrence of dead-in-shell broiler embryos

atch No.	Total No. eggs set.	No. of eggs Unhatched	Percentage	No. of dead-in-shells	Percentage
	5952	1072	18.01	578	9.71
	5334	1059	19.85	600	11.25
	6823	1298	19.02	825	12.09
	15422	2468	16.00	1542	10.00
	15449	3311	21.43	1703	11.02
	14700	3495	23.78	1342	9.13
otal	78680	16703	21.23	7796	9.91

the total isolates followed by *Salmonella* spp. 30.5 per nt (Paratyphoid salmonellae 12.7, *S. gallinarum* 11.8 and *pullorum* 5.93%) and others *Streptococcus faecalis* 5.93 r cent, *Bacillus subtilis* 4.24 per cent, *Pseudomonas ruginosa* and *Proteus mirabilis* 3.39 per cent each. These sults are completely in line with those of Sadek *et al.* 991), Alaboudi *et al.* (1992) and El-Gharib *et al.* (1993) that these workers reported the coliforms to be major oportion of dead-in-shell microflora and *E. coli* was the ain isolate among them but differs slightly in that these orkers in addition isolated *Klebsiella, Staphylococci, higella* and *Mycoplasma* which could not be isolated in the esent study. Further these results are in complete greement with Calnek *et al.* (1991) who described oliforms and *Salmonella* spp. to be the major contami-ating bacteria of hatching eggs.

able 2: Relative occurrence of various bacterial species

acteria spp.	Number of isolates	Per cent
Hemolytic	34	54.84
Non-hemolytic	28	45.16
ratyphoid salmonellae	15	12.71
lmonella gallinarum	14	11.86
lmonella pullorum	7	5.93
reptococcus faecalis	7	5.93
cillus subtilis	5	4.24
eudomonas aeruginosa	4	3.39
oteus mirabilis	4	3.39
tal	118	

high proportion of intestinal organisms particularly *E.* among the isolates suggests the faecal contamination the eggs in the breeding farms as these organisms had n isolated from egg shells and their penetration through egg shells confirmed previously (Akhtar *et al.*, 1982, cimento and Solomon, 1991). The presence of *monella* spp. Especially *S. Pullorum* and *S. Gallinarum* m for the presence of carrier birds in the breeder flock ch needs to be tested by whole blood agglutination test tandard tube agglutination test followed by retest at intervals 3-4 weeks and reactors to be removed tantly from the breeder flocks (Calneck *et al.*, 1991). 2%) of the *E. coli* isolates were found congo red +ve which suggests their pathogenic nature.

Results on the in vitro sensitivity of the bacteria from dead-in-shells to different antibiotics are presented in Table 3. The results shows multiple drug resistance among the isolates, particularly those commonly used in poultry like doxycycline ampicillin, furaltadone, erythrocyin and furazolidone to which more than 60 per cent of the isolates were resistant *in vitro* test. The results are quite comparable to those of Tariq (1989) and Baysal *et al.* (1990) who recorded multiple drug resistance among *E. coli, Salmonella* spp. and other avian isolates to the antibiotics commonly used in poultry. Neomycin, chloramphenicol and Streptopenicillin showed intermediate efficacy to which 65-68 per cent of the isolates were found sensitive but gentamicin and fluequine showed good efficacy. These results are again comparable with the above workers. Fortunately norfloxacin was found the most effective to which the in vitro sensitivity of the isolates was found to be 100 per cent. Norfloxacin is a new 4 fluroquinolone antibiotic. Its bactericidal activity in poultry is reflected from the present results.

Table 3: Antibiotic sensitivity of the isolates

Bacteria spp.	No.	Per cent
Norfloxacin	118	100.00
Gentamicin	106	89.00
Flumequine	95	80.00
Neomycin	81	68.64
Chloramphenicol	80	67.80
Streptopenicillin	77	65.00
Doxycycline	49	41.53
Ampicillin	43	36.44
Furaltadone	34	28.81
Erythromycin	17	14.41
Furazolidone	16	13.56

The overall picture of drug resistance in the bacterial isolates against the commonly used antibiotics is discouraging and gloomy. This is an indicator of misuse of drugs in poultry industry. This necessitates that the antibiotics should be used only when required in full dose and for correct duration for further medication, in vitro antibiotic sensitive tests must be performed.

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