

Aerobic Bacteria Isolated from Dead –in -Shell Chick Embryo in Khartoum State

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ملخص البحث

فحصت 540 بيضة مخصصة محتوية على أجنة نافقة، جمعت من أربع فقااسات لمزارع كبيرة بولاية الخرطوم. عزلت 212 معزولة بكتيرية تتكون من 57 (26,89%) موجبة لصبغة جرام و155 (73,11%) سالبة لصبغة جرام. شملت أجناس البكتيريا الموجبة لصبغة جرام المكورة العنقودية (19,81%) و العُصية (2,83%) والوندية (2,36%) والمكورة العقدية (0,94%) و الأركانوبياكتريوم (0,94%). أما أجناس البكتيريا السالبة لصبغة جرام فقد شملت المتقلبة (11,79%) والأشريشية (10,85%) والكلبسية (8,01%) و الزائفة (6,6%) و الليمونية (6,13%) و المتصابغة (5,7%) و اليرسينية (3,77%) و الأيرومونات (3,3%) و السالمونية (2,35%) و المقلونة (2,35%) و الصيفرية (2,35%) و المورجانية (2,35%) و السرانية (1,88%) و المعوية (1,41%) و الأيروينية (0,94%) و المفسجة (0,94%) و القوربية (0,94%) و البروفيدنيسية (0,94%) و البورديتية (0,47%). البكتيريا التي عُزلت من كل المزارع هي المكورات العنقودية و السالمونيات و الاشريشية القولونية و الكلبسيات و المتقلبات و اليرسينية الملهبة للمعى و القولون.

Summary

Five hundred and fourty dead-in-shell chick embryos were obtained from four hatcheries of big poultry farms in Khartoum state. From these, 212 bacterial isolates were isolated consisted of 57 (26.89%) Gram-positive organisms and 155 (73.11%) Gram-negative organisms. The isolated Gram-positive bacterial genera were *Staphylococcus* (19.81%), *Corynebacterium* (2.36%), *Bacillus* (2.83%), *Streptococcus* (0.94%) and *Arcanobacterium* (0.94%). The Gram-negative bacterial genera were *Proteus* (11.79%), *Escherichia* (10.84%), *Klebsiella* (8.01%), *Pseudomonas* (6.60%), *Citrobacter* (6.13%), *Chromobacterium* (5.7%), *Yersinia* (3.77%), *Aeromonas* (3.30%), *Salmonella* (2.35%), *Alcaligenes* (2.35%), *Flavobacterium* (2.35%), *Morganella* (2.35%), *Serratia* (1.88%), *Enterobacter* (1.41%), *Erwinia* (0.94%), *Janthinobacterium* (0.94%), *Plesiomonas* (0.94%), *Providencia* (0.94%), and *Bordetella* (0.47%). The organisms which were isolated from all farms were *Staphylococci*, *Salmonella*, *Escherichia coli*, *Klebsiella*, *Proteuse*, and *Yersinia enterocolitica*.

Introduction

Sudan is one of those developing countries where poultry production industry has undergone progressive development in recent years in attempts to satisfy the increasing demands for egg and poultry meat. The hatcheries of different companies in Khartoum State showed frequent reduction in hatching percentages of eggs during the year (Report of Arab- Sudanese Company, 1994–1995). The major causes of reduced hatchability were generally, adhesion, temperature variation, incubator faults (Das *et al.*, 1994), incubation humidity on the shell (Peebles, 1986), Mycoplasmal and viral infections (Stipkovits *et al.*, 1985) and bacterial infections (Ahmed *et al.*, 1981).

Bacteria represent primary or secondary agents of dead-in-shell embryos. The chick embryo itself is one of the means of disease transmission, since some pathogens are known to be transmitted transovarially (Jordan, 1979). Very little work has so far been done to study the aerobic bacteria in dead-in-shell chick embryo, despite their important role in causing death of chick embryos.

This study was carried out to investigate and find out the most important bacteria that cause death of chick embryos in local hatcheries in Khartoum State, since little information are available on the subject.

Materials and Methods

Samples were collected during September 1994 to September 1995 from hatcheries of four big poultry farms in Khartoum State. Samples were fertile eggs that were incubated for 21 days and discarded due to their failure to hatch and were considered to be dead-in-shell chick embryos. The shell was removed at the air-sack after being cleaned with alcohol. Sterile forceps and scissors were used for this purpose. A sterile loop was introduced into the egg, filled with the allantoic fluid and transferred, under the umbrella of flame, to be plated out onto Blood Agar. Isolation attempts of bacteria were made on all samples on the same day of collection. Samples were inoculated onto 10% defibrinated ovine Blood Agar and incubated microaerophilically at 37°C for 24 hours and further incubation continued for 48 hours, if no growth was evident before they were discarded as negative. All bacterial isolates were purified by frequent subculturing; pure cultures were plated out onto a separate Blood Agar plates and identified according to the procedures described by Barrows and Feltham (1993).

Results

Five hundred and forty samples were collected and investigated, from which two hundred and twelve bacterial isolates were obtained (Table 1). Isolated organisms consisted of 57 (26.89%) Gram-positive and 155 (73.11) Gram-negative bacteria (Tables 2;3;4;5).

Table 1: The number of bacterial isolates obtained from different farms

Farm	No. Tested	No. Isolates	No. +ve	No. -ve	% +ve
Arab Company for Livestock Development	120	24	23	97	19.16
African Company	150	51	48	102	32
Coral Company	150	73	71	79	47.33
Sudanese-Arabian Company	120	64	63	57	52.5
Total	540	212	205	335	37.96

No. = number; +ve = positives; -ve = negatives

Table 2: The bacteria isolated form dead-in-shell chick embryo from the hatchery of the poultry unit of Arab Company for Agricultural Production and Processing (Sudan) from September 1994 - November 1994.

No. Tested	120
No. Negative	57
No. Positive	63
% Positive	52.5

Isolates			
Gram-positive species	Isolates No.	Gram-negative species	Isolates No.
<i>Staphylococcus epidermidis</i>	5	<i>Salmonella typhimurium</i>	1
<i>Staphylococcus aureus</i>	1	<i>Pseudomonas aeruginosa</i>	5
<i>Staphylococcus gallinarum</i>	1	<i>Pseudomonas maltophilia</i>	5
<i>Staphylococcus faecalis</i>	2	<i>Pseudomonas stutzeri</i>	2
<i>Staphylococcus avium</i>	1	<i>Escherichia coli</i>	3
<i>Corynebacterium pseudodiphtheriticum</i> (C. hofmannii)	2	<i>Aeromonas hydrophila</i>	3
<i>Arcanobacterium haemolyticum</i> (formerly, <i>Corynebacterium haemolyticum</i>)	2	<i>Chromobacterium violaceum</i>	11
<i>Bacillus brevis</i>	1	<i>Janthinobacterium lividium</i>	2
		<i>Proteus vulgaris</i>	2
		<i>Proteus mirabilis</i>	1
		<i>Klebsiella oxytoca</i>	3
		<i>Klebsiella pneumoniae</i> subsp. <i>aerogenes</i>	1
		<i>Klebsiella pneumoniae</i> subsp. <i>ozaenae</i>	2
		<i>Yersinia enterocolitica</i>	1
		<i>Serratia marcescens</i>	1

	<i>Citrobacter freundii</i>	2
	<i>Enterobacter cloaca</i>	1
	<i>Plesiomonas shigelloides</i>	2
	<i>Providencia</i> sp.	1
Total Number of isolates	15	49

Table 3: The bacteria isolated form dead-in-shell chick embryo from the hatchery of Arab Company for Livestock Development from December 1994 - February 1995.

No. Tested	120
No. negative	97
No. positive	23
% + ve	19.16

Isolates			
Gram-positive species	Isolates No.	Gram-negative species	Isolates No.
<i>Staphylococcus epidermidis</i>	4	<i>Salmonella typhimurium</i>	1
<i>Staphylococcus aureus</i>	1	<i>Salmonella gallinarum</i>	1
<i>Staphylococcus avium</i>	1	<i>Escherichia coli</i>	2
<i>Corynebacterium pseudodiphtheriticum</i> (<i>C. hofmannii</i>)	3	<i>Alcaligenes faecalis</i>	2
		<i>Proteus mirabilis</i>	2
		<i>Yersinia enterocolitica</i>	2
		<i>Flavabacterium meningosepticum</i>	3
		<i>Citrobacter freundii</i>	1
		<i>Klebsiella oxytoca</i>	1
Total Number of isolates	9	15	

Table 4: Bacteria isolated form dead-in-shell chick embryo from the hatchery of African Poultry company from March 1995 - May 1995

No. Tested	150
No. negative	102
No. positive	48
% + ve	32

Isolates			
Gram-positive species	Isolates No.	Gram-negative species	Isolates No.
<i>Staphylococcus epidermidis</i>	1	<i>Salmonella typhimurium</i>	1
<i>Staphylococcus aureus</i>	1	<i>Escherichia coli</i>	10
<i>Staphylococcus gallinarum</i>	1	<i>Klebsiella oxytoca</i>	9
<i>Staphylococcus delphini</i>	2	<i>Proteus mirabilis</i>	6
<i>Streptococcus intermedius</i>	1	<i>Proteus penneri</i>	6
		<i>Enterobacter aerogenes</i>	2
		<i>Erwinia herbicola</i>	2
		<i>Yersinia enterocolitica</i>	2
		<i>Alcaligenes faecalis</i>	1
		<i>Morganella morganii</i>	3
		<i>Flavabacterium meningosepticum</i>	1
		<i>Aeromonas salmonicida</i>	1
		<i>Providencia</i> sp.	1
Total Number of isolates	6	45	

Discussion

This study is the first detailed one on aerobic bacteria isolated from dead-in-shell chick embryos and conducted on four big poultry farms in the Sudan. Previously, an attempt was made in Sudan to isolate bacteria and *Mycoplasma* from dead-in-shell chick embryos (Ahmed *et al.*, 1981). The present study was directed towards the isolation of aerobic bacteria only. The Gram-positive organisms isolated during the present work (26.89%) are less than Gram-negative bacteria (73.11%) and belong to the 5 genera; *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Arcanobacterium* and *Bacillus*. These findings were in agreement with those of Ahmed *et al* (1981). The genus *Staphylococcus* represented 18.86% of total isolates and this in agreement with Ahmed *et al* (1981), Orajaka and Mohan (1986), Wang (1992) and Alaboudi *et al* (1992). The present work shows isolation of three species, *Staphylococcus gallinarum*, *Staphylococcus delphini*, *Staphylococcus intermedius*, which were not reported to be isolated from dead-in-shell chick embryo before. Two *Streptococcus* species isolated in

this study are in accord with the result obtained by Ahmed *et al* (1981), Sezen (1985) and Alaboudi *et al* (1992). In the present study, the isolation of *Corynebacterium pseudodiphthereticum* (*C. hofmannii*) agrees with Ahmed *et al* (1981) but isolation of *Arcanobacterium* (*Corynebacterium*) *haemolyticum* was not reported before. The isolation of *Bacillus cereus* and other *Bacillus* species confirms the results of Ahmed *et al* (1981) and Lalithakunjamma and Sudharma (1991).

Table 5: Bacteria isolated form dead-in-shell chick embryo from the hatchery of Coral Farms for Chicks and Feed Production from June 1995 - August 1995.

No. Tested	150		
No. negative	79		
No. positive	71		
% + ve	47.33		
Isolates			
Gram-positive species	Isolates No.	Gram-negative species	Isolates No.
<i>Staphylococcus epidermidis</i>	3	<i>Salmonella typhimurium</i>	1
<i>Staphylococcus aureus</i>	12	<i>Escherichia coli</i>	8
<i>Staphylococcus delphini</i>	5	<i>Pseudomonas aeruginosa</i>	2
<i>Staphylococcus intermedius</i>	2	<i>Klebsiella oxytoca</i>	1
<i>Bacillus brevis</i>	3	<i>Proteus vulgaris</i>	2
<i>Bacillus cereus</i>	2	<i>Proteus mirabilis</i>	4
		<i>Proteus penneri</i>	2
		<i>Alcaligenes brochisepticus</i>	2
		<i>Flavobacterium odoratum</i>	1
		<i>Morganella morganii</i>	2
		<i>Chromobacterium violaceum</i>	1
		<i>Aeromonas hydrophila</i>	1
		<i>Aeromonas salmonicida</i>	2
		<i>Bordetella bronchiseptica</i>	1
		<i>Yersinia enterocolitica</i>	3
		<i>Serratia marcescens</i>	3
		<i>Citrobacter freundii</i>	10
Total Number of isolates	27		46

Most of the bacteria isolated in this study are Gram-negative bacteria which are consisted of 19 genera (73.11%). *Salmonellas* were isolated from all hatcheries investigated and this finding agrees with Ahmed *et al* (1981), Mazurkiewicz *et al* (1988), Alaboudi *et al* (1992) and Das *et al.* (1994). *Escherichia coli* represents 10.8% of the isolates, and was isolated from all farms investigated. This result agrees with Ahmed *et al* (1981), Stipkovits *et al* (1985), Orajaka and Mohan (1986) and Alaboudi *et al* (1992). Two species of *Pseudomonas* which were not reported before as isolates from dead-in-shell chick embryo are reported in this study as new findings concerning dead- in- shell chick embryo; these are *Pseudomonas maltophilia* and *Pseudomonas stutzeri*. Isolates of the genus *Proteus* accord with results of Ahmed *et al* (1981), Orajaka and Mohan (1986), Alaboudi *et al* (1992) and Shawabkeh and Tarazi (1993), but in the present study *Proteus penneri* is reported in dead-in-shell embryo for the first time. Other Gram-negative isolates in this study including, *Citrobacter freundii*, *Enterobacter*, *Alcaligenes*, *Erwinia herbicola*, *Chromobacterium violaceum*, *Plesiomonas shigelloides*, and *Yersinia enterocolitica*, are in agreement with those reported by Schmid *et al* (1954) and Ahmed *et al* (1981).

In the present study, six Gram-negative genera isolated from dead-in-shell chick embryos were not reported before in the Sudan. These genera are *Aeromonas*, *Morganella*, *Serratia*, *Flavobacterium*, *Bordetella* and *Providencia*.

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