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Characterization of bacteria associated with omphalitis in chicks

S. Nasrin, M. A. Islam*, M. Khatun, L. Akhter and S. Sultana
Department of Microbiology and Hygiene, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

Abstract

This study was conducted to isolate and characterize the bacteria present in cases of omphalitis in chicks. Yolk swabs (n = 60) were aseptically collected from affected chicks and cultured for isolation and identification of bacteria. *E. coli*, *Salmonella* and *Staphylococci* were identified. Bacteria were tested for sensitivity to ten common antibiotics. *E coli* isolates were sensitive to chloramphenicol and resistant to nalidixic acid, ampicillin, amoxycillin, ciprofloxacin, tetracycline, gentamicin, sulphamethoxazole and erythromycin. Salmonella were sensitive to ciprofloxacin and resistant to tetracycline. Staphylococci were sensitive to ampicillin, amoxycillin, ciprofloxacin, gentamicin, sulphamethoxazole, erythromycin, chloramphenicol and kanamycin and resistant to nalidixic acid and tetracycline. The existence of multi-drug resistance emphasises the need to prevent omphalitis in chicks by hygiene. (*Bangl. vet*. 2012. Vol. 29, No. 2, 63 – 68)

Introduction

Omphalitis is one of the leading causes of mortality in newly hatched chicks (Rahman *et al.,* 2007). It occurs due to unsanitary equipment in the hatchery. The affected chicks manifest depression, drooping of the head and huddling near to the heat source (Kahn *et al.*, 2008). Several bacteria such as *E coli., Salmonella* spp., *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Bacillus cereus* and *Enterococcus* have been isolated from the yolk sac of the infected birds (Cortes *et al.*, 2004; Iqbal *et al.,* 2006).

Early and accurate detection of bacteria is important to undertake appropriate control measure. Good management and sanitation as well as use of antibiotics help reduce mortality. The objectives of this study were to isolate the bacteria from clinical cases of omphalitis in newly hatched chicks in two hatcheries and to determine their sensitivity to ten common antibiotics.

Materials and Methods

Collection of samples

Yolk swabs (n = 60) were aseptically collected from newly hatched chicks at Bogra and Bangladesh Agricultural University (BAU) hatcheries manifesting signs of

*Corresponding author:- *E-mail*: arifmicro2003@yahoo.com

omphalitis. At Bogra, 15 samples were collected from chicks 1-3 days old and 27 from chicks 4-7 days old. At BAU hatchery, ten samples were collected from chicks 1-3 days old and 8 from chicks 4-7 days old. Samples were transported to the laboratory at 4°C.

Isolation of bacteria

Samples were enriched by overnight incubation in nutrient broth at 37°C. Cultures were inoculated onto nutrient agar (NA), blood agar (BA), eosin methylene blue (EMB) agar, brilliant green agar (BGA), mannitol salt agar (MSA), salmonella-shigella (SS) agar and triple sugar iron (TSI) agar and incubated at 37°C. Discrete bacterial colonies were sub-cultured until pure cultures were obtained (Cheesbrough, 1985).

Characterization of bacteria

Bacteria were characterised by recording morphology of colonies (size, margin, elevation and colour), Gram stain (Merchant and Packer, 1967), sugar fermentation, catalase, coagulase, M-R, V-P, indole, and triple sugar iron tests (Cheesbrough, 1985).

Antibiotic sensitivity

Antimicrobial sensitivity was tested using 0.5 McFarland turbidity standard inoculum and freshly prepared, dried Mueller Hinton agar (Oxoid, UK) against 10 common antibiotics: nalidixic acid, ampicillin, amoxycillin, chloramphenicol, ciprofloxacin, tetracycline, kanamycin, gentamicin, sulphamethoxazole and erythromycin (Oxoid, UK). Five isolates of *E. coli*, *Salmonella and Staphylococci* were selected randomly for the test. Disc diffusion or Kirby-Bauer method (Bauer *et al.,* 1966) was used. The results were expressed as resistant, intermediate or sensitive according to the guidelines of National Committee for Clinical Laboratory Standards (NCCLS, 2007).

Results and Discussion

Isolation of bacteria

Three genera of bacteria were isolated from yolk swab samples of chicks, *E. coli, Salmonella* and *Staphylococc*i (Table 1). Bacterial genera recovered are in agreement with earlier studies (Sato *et al.,* 1961**;** Zahdeh *et al.,* 1984; Ijaz *et al.,* 1994**;** Munir *et al.,* 2004; Iqbal *et al.,* 2006).

Table 1. Bacteria isolated from yolk swabs of chicks suffering from omphalitis in Bogra and BAU hatcheries

Hatchery	Chicken age	No of samples	No of bacterial isolates		
			E. coli	*Salmonella*	*Staphylococci*
Bogra	1-3 days	15	8	13	5
	4-7 days	27	15	18	7
BAU	1-3 days	10	4	3	1
	4-7 days	8	1	1	3

BAU = Bangladesh Agricultural University

Prevalence of bacteria

The prevalence of bacteria associated with omphalitis in chicks is presented in Fig. 1.

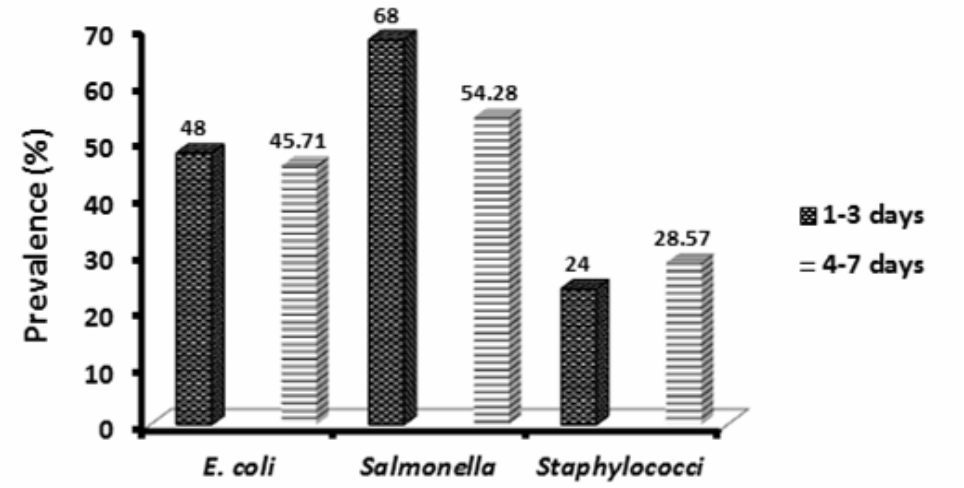

Fig. 1. Prevalence of *E. coli*, *Salmonella* and *Staphylococci* in 1-3 days and 4-7 days old chicks with clinical signs of omphalitis

In this study *Salmonella* showed the highest prevalence both in chicks aged 1-3 days and 4-7 days (68 and 54.3%, respectively). These findings contradict the observation of Iqbal *et al.* (2006) who recorded a prevalence of *E. coli* 47.9% and only 0.5% prevalence of *Salmonella*. The prevalence of *Staphylococci* ranked third in this study (24% in 1-3 days old chicks and 28.6% in 4-7 days old chicks), but a previous study recorded 0.5% prevalence of *Staphylococci* (Iqbal *et al.*, 2006).

Cultural, morphological and staining characteristics

The cultural characteristics of *E. coli*, *Salmonella* and *Staphylococci* (Table 2) were similar to the findings of other authors (Choudhury *et al.*, 1993; Nazir, 2004; Jakaria *et al.*, 2012; Naurin *et al.*, 2012).

Biochemical characteristics

E. coli fermented dextrose, lactose, sucrose and mannitol with the production of acid and gas. *E. coli* gave positive reaction to catalase and MR and indole tests and negative reaction in V-P test. *Salmonella* fermented dextrose, maltose and mannitol with acid and gas production. *Salmonella* were MR and catalase positive and negative to V-P and indole tests. *Staphylococci* fermented all five basic sugars with only acid production. Catalase, MR and V-P tests were positive but indole and coagulase tests were negative. These results are similar to those of Sato *et al.* (1961); Zahdeh *et al.* (1984) and OIE (2004).

Table 2. Cultural characteristics of bacteria isolated from yolk swab samples of chicks suffering from omphalitis

Bacterial isolates	Cultural characteristics on						
	NA	EMB agar	BA	BGA	MSA	SS agar	TSI agar slant
E. coli	Smooth, circular, white to greyish white colony	Large, circular, blue-black colony with green metallic sheen	Colourless colony without haemolysis	Yellow colour colony	No growth	Slight growth and pink to rose-red colony	Yellow slant and butt with gas but no H_2S production
Salmonella	Circular, smooth, opaque and translucent	Pink colour, circular and smooth colony	Non-haemolytic colony	Pale pink colour colony against a pinkish background	No growth	Black centred, smooth, small round colony	Butt remains yellow and slant converted to pink colour
Staphylo-cocci	Round, flat colony of sticky, mucoid consistency	No growth	Round, greyish and mucoid colony without haemolysis	No growth	Small grey-white or yellowish colony	No growth	No growth

NA = nutrient agar; EMB = eosin methylene blue; BA = blood agar; BGA = brilliant green agar; MSA = mannitol salt agar; SS = salmonella-shigella; TSI = triple sugar iron. *E. coli and Salmonella* were Gram-negative, small rod-shaped single or paired. *Staphylococci* were Gram-positive, rod-shaped and arranged in clusters, in agreement with Freeman (1985) and Jones *et al.* (1997).

Antibiotic sensitivity

The results are presented in Table 3.

Table 3. Antibiotic sensitivity of *E. coli, Salmonella* and *Staphylococci*

Antimicrobial agents	Disc concentration (µg/ml)	*E. coli* (n = 5)			*Salmonella* (n = 5)			*Staphylococci* (n = 5)		
		R	I	S	R	I	S	R	I	S
Nalidixic Acid	30	5	0	0	4	1	0	5	0	0
Ampicillin	10	5	0	0	2	0	3	0	1	4
Amoxicillin	10	5	0	0	3	0	2	0	1	4
Chloramphenicol	30	0	0	5	2	1	2	0	1	4
Ciprofloxacin	5	5	0	0	0	0	5	0	0	5
Tetracycline	30	5	0	0	5	0	0	5	0	0
Kanamycin	30	1	2	2	1	2	2	2	0	3
Gentamicin	10	5	0	0	0	3	2	0	1	4
Sulphamethoxazole	25	5	0	0	3	0	2	2	0	3
Erythromycin	15	5	0	0	5	0	0	1	2	2

R = resistant; I = intermediate; S = sensitive

All *E. coli* isolates were resistant to eight antibiotics: ciprofloxacin, gentamicin, amoxycillin, ampicillin, tetracycline, erythromycin, nalidixic acid and sulphamethoxazole. All *Salmonella* isolates were resistant to tetracycline and erythromycin. All *Staphylococci* were resistant to nalidixic acid and tetracycline. The results are identical with those by Klein *et al.* (1996); Khan *et al.* (2002); Lee *et al.* (2005); Nazir *et al.* (2005a, b); Akond *et al.* (2009).

Conclusions

The occurrence of multi-drug resistance in bacteria in chicks suffering from omphalitis is alarming as this resistance may gain access to man and animals, which might result in difficulties in treatment of bacterial infection. Further studies are required to formulate guidelines for the prevention and control of bacterial omphalitis in chicks in Bangladesh.

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