Vet.Med.J.,Giza. Vol.42,No.1.(1994):277-285

STUDIES ON MAJOR BACTERIAL AGENTS CAUSING ARTHRITIS IN CHICKENS IN KALUOBIA PROVINCE.*

BY

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Received : 29 / 1 / 1994

SUMMARY

Twelve broiler farms and one hatchary were examined for presence of arthritis in Kaluobia Province. The morbidity rate ranged from 0.5 % in baby chicks to 20% in 22 day old chicks with an overall incidence of 8.35%.

The higher morbidity was detected in 18-28 day old chicks. The bacterial affections raged form 8 -26.31 % except in baby chicks reach 60%. 129 bacterial isolates were recovered from the joints and internal organs of 47 birds. All strains were subjected to specific biochemical tests according to the morphological and staining characters. Staphylococci was isolated in a percentage of (28.7 % followed by salmonellae (24.8%); *E. coli* (19.4%) mycoplasma (17.8%), streptococci (8.5%), then corynebacterium (0.78%).

Serological tests were done to some types (acording the availability of antisera). Thirteen strains of *E. coli* were typed as O 114: K; I 128: K67 and O 119. K69 serotypes. Thirty - two salmonellae were typed, 20 as Poly 1 and 12 as poly I & II. Twenty three Mycoplasma isolates were typed by growth inhibition test as *Mycoplasma Gallisepticum* (MG) by using specific antisera.

Four out of 60 arthritic birds, were positive for *MG* (6.6%) recovery, and 8 birds (13.3%) had antibodies titer against *MG* and *MS* in a percent of (87.5% and 12.5%) respectively

INTRODUCTION

Arthritis is one of the major problems facing the chicken breeders resulting in decreased body weight, emaciation and / or deaths. Gross (1961) was the first who recovered *E. coli* O-group 15 from 5- week-old white Rock poults suffering from synovitis, followed by Janovski (1966) in caged birds, Narin (1973), in turkeys, Gordon & Jorden (1982), and Gross (1991). Mycoplasma could be reported by several investigators as a cause of arthritis (Lecce, 1960; Chaiquest and Fabricant, 1960; Olson et al., 1963; Kerr and Olson, 1964; Lorna Timmes, 1966; Olson and Kerr, 1967; Olson, 1972; King et al., 1973; Olson and Sahu, 1975; Cole et al., 1965; El-Shabiny et al., 1990 and Morrow et al., 1990). *Salmonells pullorum* was isolated from chicks with arthritis by Durant and McDougle (1932); Beaudette (1936); Reis (1942); Prier and Rhoades (1948); Carnaghan and Sojka (1958) and Ferguson et al., (1961).

Salmonella typhimurium was recoverd from chicks with arthritis by Mario Padron (1989). *Staphylococcus aureus* proved to be a cause of arthritis in chickens by Wang et al. (1977). Bergmann et al., (1980) Kohler et al., (1980), Mutalib et al., (1982); Griffiths et al., (1984); Hill et al. (1988) and Milakovic et al., (1989).

* Part of thesis submitted to the faculty of Vet. Medicine for the degree of M. V. Sc. Zagazig University Benha branch (1993).

Streptococcus zooepidmicus was recovered from chickens with arthritis by Buxton (1952); Peckham (1966); Gross (1984); and Wages (1991). So, the present study is dedicated to throwa beam of light on causes of arthritis in broiler chickens in Kaluobia province, especially those due to bacterial agents.

MATERIAL AND METHODS

Media: Nutrient agar, sheep blood agar 5%. MacConkey broth, MacConkey agar, Desoxycholate citrate agar (for isolotion & differentiation of Enterobacteriaceae) Eosin methylene blue agar (EMB) for *E. coli* PPLO media for mycoplasma. (Hayflick; 1965). Frey's media for *Mycoplasma synoviae* (Frey et al., 1968) Heart infusion agar & broth (Sabry, 1968) Baird Parker media, for differentiation between pathogenic & nonpathogenic staphylococci. (Baird-Parker, 1962). Kenner Fecal (KF), streptococcal agar media for streptococci, (Kenner at al., 1961).

Stains: Gram stain, Lishman's and Giemsa stains.

Diagnostic antisera, antigens and strains:-

Salmonella test sera (poly I and poly I & II) and *E. coli* agglutinating antisera (Pool A, B & C) and monovalent ok antisera were Purshased from Behring Paul-Ehrlich Institute, Fedral office for sera and vacines (Germany).

My-Coplasma gallisepticum antisera & Reference strains PG 31 & S6 Were supplied by Animal Health Research Institute, My-coplasma Department, Dokki, Egypt.

Mycoplasma gallisepticum and *Mycoplasma synoviae* stained antigens were supplied by Vet-International B. X. Boxmer, Holland. Hemagglutination-Inhibition antigen according to Sabry (1968) was supplied by Animal Health Research Institute, Mycoplasma Department, Dokki, Egypt.

Isolation and purification of aerobic bacterial isolates:- Sacrificed birds and/or freshly dead ones were soaked in a disinfectant prior to removal of the skin.

Swabs from the joint contents were taken aseptically. The skin was removed followed by opening the obdy cavity aseptically, loops were taken from the liver, spleen, heart blood, lung, air sacs and yoik sac (if present), and streaked on nutrient agar, blood agar and MacConkey agar media.

For primary my-coplasma isolation two types of materials were used joint exudate (if any) and supernatant tissue emulsion. Each sample was streaked onto PPLO agar & Frey's agar, and also immersed into PPLO broth and Frey's broth incubated for 3 successive days at 37°C under humidity & 10% Co2 tension.

Suspected broth cultures were re-inoculated on mycoplasma broth and mycoplasma agar media. Cultures were not considered negative except after 3 weeks observation period.

Maintenance of mycoplasma cultures I Agar block containing colonies, incubated for 2 dyas at 37°C then frozen at -20°C.

Purification of mycoplasma broth culture:-Serial tenfold dilution from broth culture were made. 0.02 ml was taken from each dilution & cultured on specific media, incubated for 3 days at 37°C . The plates were examined under dessecting microscope for the presence of well separated mycoplasma colnies. Up to three replicates was done till pure cultures were obtained.

*Identification:-*This was done according to the cultural and stai-ning characters. Table 1 and 2 summarizes the biochemical and sugar fermentation reaction according to the character of the isolate. (Cruickshank et al., (1975); Macfaddin, 1980); Bergy's,(1983); Koneman et al., (1983); Carter and Chenogappa (1990).

Serological identification for *E. coli.* This was carried out according to Hallmann and Burkhardt (1974): Slide agglutination test, using polyvalent antisera then by mono specific antisera. The O-groups of *E.coli* were identified by tube agglutination test. For mycoplasma: 1-Growth

[illegible] test according to Clyde (1964). The test was done by the rotating drop technique.

B- Serodiagnostic procedures for detection of antibody against *MG* and *MS*. Slide serum plate agglutination test (SPA), and Hemagglutination inhibition test (HI) were doen according to Messaros, (1964)

For salmonella, Slide agglutination test: (using polyvalent O & H antisera) for the demonstration of somatic and flagellar antigens of biochemically identified strains.

RESULTS

Occurance & incidence of arthritis: Two hundred and fifty five arthritic birds representing 12 chickens farms and one hatchary were examined bacteriologi-cally. The percentage of bacterial infection ranged from 8.00-26.31% except in baby chicks (hatchary chicks) it reached 40% (Table 3).

positive cocci short chain like; one isolate was gram positive bacilli chinese letter in arrangement. Twenty-three mycoplasma like colonies on mycoplasma agar plates (seen by dessecting microscope). Coccoid microorganism were seen in smears from mycoplasma colonies stained by Giemsa stain. Table 5 ummarizes the distribution of the different bacterial agents and their percentages in the different organs of the 47 bacterologically positive birds.

Biochemical tests.

Biochemical tests, for lactose fermenting & non lactose fermenting colonies, corynebacterium colonies, suspected mycoplasma isolates, suspected staphylococcal colonies, and suspected streptococcal colonies (were done according to Cruicksh-ank et al., (1975).

Serotyping of some of the biochemically identified microorganisms. *E. coli serotyping*. Seven isolates were typed as 0114: K; 4 as 0128: K67. and 2 as 0.119: K69 serotype. The other 12

Table [illegible] Biochemical reactions in association with the type of isolate.

Type of isolate	Arginine test	Sugar test	Glucose splitting test	TSI red test	Growth on 6.5% NaCl	Catalase test	Oxidase test	Indole test	Nitrate red test	Methyl red test	VP test	Citrate test	Urease test	H_2S prod. test	Sucrose 6.5% test	Coagulase test	Novobiocin test
[illegible]	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	+
[illegible]	-	-	-	-	-	-	-	+	-	+	+	+	+	+	-	-	-
[illegible]	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
[illegible]	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-
[illegible]	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+	-
[illegible]	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-

[illegible]
[illegible]
[illegible]
[illegible]

Clinical and post-mortem manifestations: The specific clinical signs and post mortem findngs are presented in (Table 4).

Bacteriological identification.

Morphological studies: A total 129 bacterial isolates were differentiated. Twenty-five isolates were gram negative, lactose fermenting organisms, 32 were gram negative, non lactose fermenting organism; 37 were gram positive cocci, cluster like in appearance of which some colonies were hemolytic; 11 isolates were gram

isolates were untyped by the available antisera. (Table 7).

Salmonella serotyping: Twenty isolates were typed as poly I; and twelve isolates were typed as poly I and II. (Table 8).

-Mycoplasma serotyping.

Mycoplasma serotyping by growth inhibition test, are presented in (Table 6). Detection of mycoplasma antibodies in the sera of chickens as well as cultivation was clear in (Table 9).

Table (2): Sugar reaction in association with the type of isolate.

Type of isolate	Glucose	Lactose	Maltose	Mannitol	Dulcitol	Dextrose	Salicin	Sucrose	Sorbitol	Inositol	Dextrin	Arabinose
E. Coli	+	+	+	+	+	--	+	+	+	+	+	+
Corynbact.	+	--	--	+	--	--	+	+	--	--	--	--
Mycoplasma	+	--	--	--	--	--	--	--	--	--	--	--
Salmonella	--	+	+	+	+	+	+	+	+	+	--	--
Staphylococci	+	--	+	+	--	--	+	--	--	--	--	--
Streptococci	+	--	--	+	--	--	--	--	--	--	--	--

+ = was done
-- = was not done

Table (3: The occurence and incidence of arthritis in chickens in Kaluobia province.

Flock number	Farm locality	Age of birds in days	Total number birds	Number of clinically sick birds	Incidence of sick (%)	No. of examined birds	Bacteriologically positive birds	Incidence of positive (%)
1	Moshtohor	35	5000	200	4	15	3	20
2	Moshtohor	45	5000	100	2	13	2	15.38
3	Shiblanga	20	5000	500	10	23	4	17.19
4	Kauffer-Awis	22	5000	1000	20	24	6	25
5	Shiblanga	26	5000	500	10	26	4	15.18
6	Toukh	30	4000	75	1.8	17	3	17.65
7	Kauffer-Awis	18	5000	500	10	25	4	16
8	Toukh	28	5000	700	14	18	3	16.66
9	Benha	20	5000	750	15	22	4	18.18
10	Eldair	35	5000	200	4	23	4	17.39
11	Moshtohor	40	4000	150	3.7	19	5	26.31
12	Moshtohor	28	5000	250	5	25	2	8
13	Sinhera Hatchary	4	1000	5	0.5	5	3	60
Total		---	59.000	4930	8.35	255	47	18.43

Table (4): Clinical and post mortem examination of the affected birds.

Affected birds (flock number)	Clinical examination									Post mortem examination														
	Dro.	O.F.	R.F.	Dia.	N.D.	F.Co	C.Co	Lam.	S.J.	Per.	Myd. & A.	G.L.	C.L.	N.L.	Peri	C.S.	N.S.	S.J.	C.La	N.D.	C.K.	U.U	Ent.	J.E.	U.Y.
1	+	+	+	+	+	+	-	+	+	+	-	-	-	+	-	+	-	-	+	+	-	-	+	+	-
2	+	+	+	-	-	+	-	+	+	-	-	-	+	-	-	+	-	+	+	+	+	+	-	+	-
3	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
4	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
5	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
6	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
7	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
8	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
9	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
10	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
11	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
12	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
13	+	+	+	-	-	+	-	+	+	-	-	+	-	+	-	+	-	+	+	+	+	-	+	+	-
Total	13	13	13	4	6	10	3	13	13	6	3	6	8	9	5	11	5	5	10	8	9	7	8	13	2
%	100	100	100	30.8	46	77	23	100	100	46.2	23	46.1	61.5	69.2	38.4	84.6	38.4	38.4	77	61.5	69.2	54	61.5	100	9

Dro. = Droopiness
O. F. = Off food
R.F. = ruffled feathers
Dia. = Diarrhoea
F. CO= Paleness of comb
Lam.= Lameness
Peri = Pericarditis

Per. = Pericarditis
Myd. & A. = Hydropericardium and Ascites
G. L. = Greenish liver
C. L. = Congested liver
N. L. = Necrosed liver
C. Co.= Congested comb
S.J. = Swollen joint
C. S. = Congested spleen

C. K. = Congested kidney
N. S. = Necrosed spleen
C. La = Congested lung
N. D. = Nasal discharge
U. U. = Urate in ureters
Ent. = Enteritis
J. E. = Joint exudate
U. Y. = Unabsorbed yolk

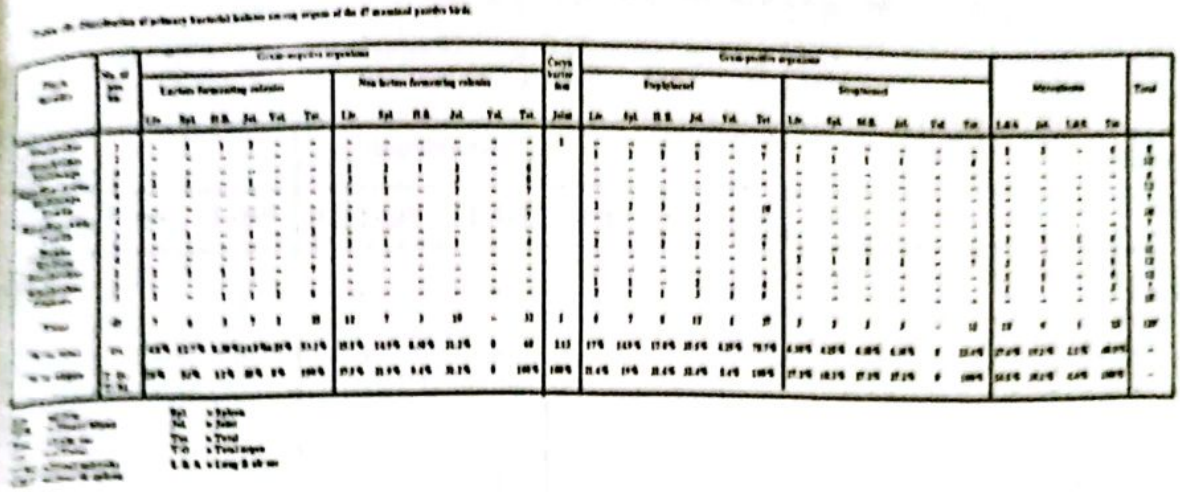

Table (6): Tests used for identification of the suspected Mycoplasma colonies.

No. of positive / No. of examined	No. of the isolates	Digitonin test	Biochemical test			Serotyping by GI test
			glucose	arginine	TTC	
10/255	23	+	+ A	--	+ red	+

* after (cruiekshank et al., (1975).
-- = negative results
+ = positive results
TTC = 2,3,5, Triphenyl tetrazolium chloride reduction test
GI = growth inhibition test
A = acid production.

Table (7): Serological identification of *E. coli* isolates.

Locality	Total no. of examined strains	*E. coli* serotypes					Total typed	Serotype
		Liver	Spleen	heart blood	Joint	Yolk sac		
Moshtohor	4	--	1	1	1	--	3	0114:K-
Toukh	3	--	1	--	1	--	2	0119:K69
Kauffer Awis	5	1	2	--	1	--	4	0114:K-
Moshtohor	7	--	--	--	--	--	--	--
Sinhera	6	--	--	1	1	2	4	0128:K67
Total typed		1	4	2	4	2	13	

Table (8): Serological identification of the motile salmonellae isolates.

Locality	Salmonella types							
	Liver		Spleen		Heart blood		Joint	
	Poly I	Poly I & II	Poly I	Poly I & II	Poly I	Poly I & II	Poly I	Poly I & II
Shiblanga	--	2	2	--	--	--	2	1
Kauffer Awis	2	1	1	--	1	1	2	
Shiblanga	1	1	2	--	--	--	1	
Kauffer Awis	1	2	--	1	1	--	2	
Benha	2	--	--	1	--	--	--	2
Total		6	5	2	2	1	7	3

Table (9): Results of mycoplasma cultivation and silde serum plate agglutination test (SPA) and hemagglutination inhibition test (III).

No. of examined birds		No. of postive sera or culture	%	SPA + for				SPA + for*	
				MG	%	MS	%	MG	MS
Serum	60	3	13.3	7	87.5	1	12.5	7	1
Culture	60	4	6.6	--	--	--	--	--	--

iters of 1: 40-1 80 in MG and only 1 : 40 for MS
Mycoplasma gallisepticum isolation is described in table (5).

Table (10): Overall percentage of different microorganisms recovered from 255 arthritic birds.

%Type of organism	No. of isolates	Incidence of isolation of each type of orgainsms
E. coli	25	19.4%
Corynbacterium	1	0.78%
M. gallisepticum	23	17.81
Salmonella (motile) poly I & poly I & II	32	24.81%
Staphylococci	37	28.7%
Streptococci	11	8.5%
Total	129	100%

Incidence of microorganisms among arthritic birds: From (Table 10), it is evident that staphylococci (28.7%) was the most frequently isolated microorganisms followed by almonellae (24.81%); *E. coli* (19.4%); mycoplasmas (17.8%); streptococci (8.5%); and corynebacteria (7.76%) subsequently.

DISCUSSION

Forty seven out of two hundred and fifty five birds (collected from 12 broiler farms and one hatchary) with arthritis gave positive results on bacteriological examination (18.43%).

The percentage of arthritis ranged from 1.8 to 20% in birds aging eighteen to forty five days old, while it was only 0.5% in younger chicks. The lower percentage of arthritis in baby chicks was obtained from examination of one hatchary. The authors could not avoid this defect because nearly all the owners of the hatcheries did not allow them to examine their newly hatched chicks.

The clinical signs observed in affected birds were in agreement with those of Prier and Rhoades (1948); Cover et al., (1956); Sevoian et al., (1958); Ferguson et al., (1961); Janovski (1966) Lorna Timmes (1966); Peckham (1966); Jordan (1979); Gross (1984); Cloe et al., (1985); Mario Padron (1989); and Wages (1991). Joint swelling with exudate was a common finding in all examined birds. Most birds showed congestion in the spleen, liver, lungs, Kidneys, sometimes with necrosis in the liver and spleen and urates in ureters. The observation agrees with those of Beaudette (1936); Prier and Rhoades (1948); Cover et al., (1956); Carnaghan and Sojka (1958); Ferguson et al., (1961); Janovski (1966); Loma Timms (1966) Olson (1972) and Jordan (1979).

There were additional findings on some necropsied birds such as pericarditis, perihepatitis, hydropericardium and unabsorbed yolk sacs (if any). These findings were also reported by Ferguson et al., (1961) Colusi and Sequeira (1964); Gross (1984); Mario Padron (1989) and Wages (1991).

The authors could not observe the gross lesions that were reported by Gross (1961) Who found large caseous masses in the left kidney and in the liver in addition to synovitis, and that of Janovski (1966) who observed crippling inflammation of the joints that lead to gangrene of the phalanges and tarsophalangeal joint in caged birds.

Bacterial isolation was higher in baby chicks than aged birds; 60% versus 28.3% (Table 3). This may be due to bacterial infection in or/on the eggs during incubation, and there was no antibiotic treatment according to the owners history Furthermore, obtained baby chicks may be all culls from the hatchary. The lower incidence in aged birds may be due to either complete recovery through antibiotic usage or mortality of seriously affected birds. Also older ages there were continuous adminstration of antibiotics (tetracycline, chloramphincol, oxytetracycline, furazolidone streptomycin and erythromycin) according to the owners history. Twenty five *E. coli* strains were recovered; 7. Strains were typed as 0114:K1, 4 as 0128: K67 and 2 as 0119:K69 serotypes. The joints was the most common site in isoltation followed by the spleen, heart blood and liver respectively. Gross (1961) was the only one who isolated *E. coli* O-group 15 from the kidney of 4-weeks-old white rock poults suffering from synovitis, while Janovski (1966), and Narin (1973) recovered different *E. coli* serogroups from caged birds & turkeys suffering from synovitis.

Thirty-two motile salmonella isolates could be typed; 20 as poly I and 12 as poly I & II. The liver was the common organ in isolation of salmonella followed by Joint tissues, spleen and heart blood respectively. This agrees only with the report of Mario Padron (1989) who isolated *Salmonella typhimurum* from internal organs eyes and hock joints of diseased chickens. Several investigators isolated *Salmonella pullorum* (non motile) from chickens affected with arthritis as Durant and McDougle (1932) Beaudette (1936); Reis (1942) Carnaghan and sojka (1958); Ferguson et al., (1961), and Colusi and Sequeira (1964). Twenty three isolates of *MG* were typed by growth inhibition test. They were isolated mostly from the lung and air sacs (56.52%) followed by the joints (39.13%) and liver (4.35%). Other investigators recovered *MG* from cases of swollen joints (Lorna Timms, 1966; Cole et al., 1985 and El-Shabiny et al., 1990).

Unfortunately, *MS* could not be recovered in this investigation inspite of the detection of its antibodies inonly one serum sample. This may be due to the long continious adminstration of drugs and / or the explanation of Kerr and Olson (1967). who found that isolation from the joint and organs appeared to be related to resistance it was difficult or sometimes possible to isolate the organism when resistance completed.

MS could be recovered from arthritis by Jordan (1979), Cole et al. (1985), El-Shabiny et al., (1990) and Morrow et al., (1990). Sixty serum samples were collected randomly from chickens aged 28-40 days old. They were subjected to bacteriological as well as serological examination (slide plate agglutination and hemagutination inhibtion test subsequently) for Mycoplasmas. Only eight samples (13.3%) gave positive results, seven of them were positive toward *MG* antigen (87.5%) and only one sample was positive toward *MS* antigen (12.5%). Some birds (6.6%) gave both positive results on bacteriological as well as serological examination for *MG* which may be due to the presence of drug resistant strains inspite of drug adminstration. This finding partially agrees with that of El- Shabiny et al., (1990). Staohylococci was the higher in the percentage of isolation 28.7% (18.92%) were coagulase positive and (81.08%) were coagulase negative. These findings partially agrees with that of Kohier et al (1980). Corynebacteirum was isolated from the joints of a broiler chick. To the best of our knowledge there was no isolation of this organism from the joints of birds at least in Egypt. This may be due to abrasion or wound infection in the joints from contaminated straw or hay.

Conclusion: From the above study it can be concluded that staphylococci was the most recovered microorganisms (28.7%) from arthritic birds followed by Salmonellae (24.8%), *E. coli* (19.4%), mycoplasma (12.8%), Streptococci (8.5%) and Corynebacterium (0.78%).

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