Prevalence of Different Bacterial Isolates Recovered from Broiler Chicks Suffered from Respiratory Manifestations Associated With Diarrhea

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Abstract

The respiratory system serves as the principal site of infection for many pathogens that cause chronic diseases in birds, and respiratory problems are the primary cause of financial losses in the poultry industry. This study was aimed to isolate different bacterial pathogens affected broiler chicks. Samples were collected from freshly dead and diseased chicks that suffered from respiratory manifestations associated with diarrhea. These samples under test (n=6433, 4628, 4613, 6377 and 4713) for isolation of *Salmonella, E. coli*, *Klebsiella*, *Proteus* and *Pseudomonas*, respectively. Samples were collected from broiler chicks aged from one to 35 days were collected from Wahat, Regwa (Cairo 3 A company) and external farms, Egypt. The prevalence of isolated bacterial pathogens were 9%, 65.3%, 8.8%, 1% and 3.2% for *Salmonella*, *E. coli*, *Klebsiella*, *Proteus* and *Pseudomonas*, respectively. All the isolated bacterial pathogens identified biochemically by using Indole test, Methyl red test (MR), Voges-Proskauer test (VP), Citrate utilization test, Catalase test, Oxidase test, Hydrogen sulphide production test, Urease production test, Sugar fermentation, nitrate reduction test, triple sugar iron agar and Lysine decarboxylase test.

Keywords: *Salmonella, E. coli, Klebsiella, Proteus, Pseudomonas*

Introduction

Bacterial diseases of poultry have to be routinely surveyed and monitored due to they are sources of foodborne diseases, have emerging public health concerns about antimicrobial use and finally affect animal health and critical importance economic losses (***Agnes et al., 2012*****).** *Salmonella enterica* subspecies *enterica* serovars and *E. coli* not only jeopardize poultry industry but they are of potential zoonotic significance. *Salmonella* spp. could reach human via diverse ways including direct contact and food chain. In the USA, a total of 53 documented outbreaks of live poultry-associated salmonellosis (LPAS) were reported during 1990-2014 and they included 2,630 illnesses, 387 hospitalizations, and five deaths **(*Basler et al., 2016*)**. *Proteus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp., *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Bacillus cereus, E. coli*, *Salmonella* and *P. aeruginosa* were bacteria that were recovered from chicks in different places globally **(*Ulmer,* 2011).** *Klebsiella pneumoniae* is a prevalent infectious illness that affects chicks and causes significant economic losses **(*Aly et al., 2014*).** The respiratory illness is characterized by dyspnea, pump handled respiration, gasping, mucous discharge, facial edema, sinus enlargement, tracheitis, exudative pneumonia, pleuritis, air saculitis, pericarditis, reduced egg production, and low egg quality **(*Tantawy et al., 2018*).**

P. aeruginosa is an opportunistic pathogenic bacterium responsible for serious problems in poultry farms. It considered as a good example of environment associated bacteria. Besides its natural resistance to many antimicrobial substances and traditional disinfectants, it has ability to form biofilm (***Maram et al., 2018*****).** Aim of work: investigation of the high-risk bacterial pathogens that impact broiler chicks.

Material and Methods

Statement of Ethical Considerations

In the current study followed ARRIVE guidelines. The Animal Ethics Review Committee at Suez Canal University (AERC-SCU), Egypt, endorsed all broiler chicks handling and experiments.

Sampling:

A total number of (n=6433, 4628, 4613, 6377 and 4713) for isolation of *Salmonella, E. coli, Klebsiella, Proteus* and *Pseudomonas,* respectively. Were collected aseptically from heart, lung, bone marrow, brain, cecai, fish meal, gizzard, liver, kidney, proven, trachea, yolk sac, intestine, drags from litter, box linner, organs and ration) freshly dead and diseased chicks that suffered from

respiratory manifestations associated with diarrhea and/or freshly dead chicks collected from Wahat, Regwa (Cairo 3 A company) and external farms, Egypt as shown in Table (1) and Figure (1).

Isolation of different bacterial pathogens:

For isolation of *Salmonella,* all samples underwent a pre-enrichment step in nutrient broth (Oxoid, USA). Then samples were incubated aerobically for 24 hours at 37 ºC for 18 h then the pre-enriched media were inoculated into enriched media (Rappaport vassiliadis soya broth) and incubated aerobically at 37ºC for 16 h and incubated aerobically at 37ºC for 16 h after that loopful from the enriched media was streaked onto XLD (Oxoid, USA). for isolation of *E. coli, Klebsiella, Proteus*, samples were incubated aerobically for 24 hours at 37 ºC for 18 h then the pre-enriched media were inoculated into enriched media MacConkey broth medium (Oxoid, USA) for isolation of the reminder bacterial pathogens and finally loopful from the enriched media was streaked onto MacConkey agar medium (Oxoid, USA) as selective media for isolation of *E. coli, Klebsiella, Proteus* and loopful from the enriched media was streaked onto Cetrimide agar base (Oxoid, Uk) as selective media for isolation *Pseudomonas*.

Biochemical identification of the different bacterial species:

All the isolated bacterial pathogens *Enterobacteriacae* (*Salmonella, E. coli, Klebsiella* and *proteus*) and *Pseudomonas* identified biochemically according to (***Cruickshank et al., 1975*; *Collee et al., 1996***) by using Indole test, Methyl red test (MR), Voges-Proskauer test (VP), Citrate utilization test, Catalase test, Oxidase test, Hydrogen sulphide production test, Urease production test, Sugar fermentation and Lysine decarboxylase test and nitrate reduction test.

Results

Prevalence of the different bacterial species:

Prevelence of the isolated bacterial pathogens from chicks manifested respiratory signs associated with diarrhea during 2021:

The prevalence of isolated bacterial pathogens during 2021 which were (8.5%, 62.4%, 7.7%, 1.7% and 2.4%) for *Salmonella, E. coli, Klebsiella, Proteus* and *Pseudomonas,* respectively as shown in Table (1) and figure (1)

Prevelence of the isolated bacterial pathogens from chicks manifested respiratory signs associated with diarrhea during 2022: The prevalence of isolated bacterial pathogens during 2022 which were (9.7%, 69.8%, 10.5%, 0% and 4.4%) for *Salmonella, E. coli, Klebsiella, Proteus* and *Pseudomonas,* respectively as shown in Table (2) and figure (2)

biochemical identification results: Suspected *Salmonella* isolates were all positive for citrate utilization, methyl red, hydrogen sulphide production on TSI agar (Oxoid, USA) and nitrate reduction test. They were negative for indole production, V.P, lactose fermentation and urea hydrolysis tests. The isolated *Salmonella* isolates fermented arabinose, glucose, maltose, mannitol, mannose and sorbitol. While The *E. coli* isolates were all negative for citrate utilization, urea hydrolysis test and V.P. They were all positive for indole production, methyl red and fermentation test of arabinose, glucose, lactose, maltose, mannitol, and mannose while they gave variable results with sorbitol and sucrose. Additionally, all isolates were actively motile in semi-solid agar. Additionally, *E*. coli showed acid production within both butt and slant of TSI agar represented by yellow colour without hydrogen sulphide production. But *klebsiella* isolates were all negative for oxidation indole, methyl red and No hydrogen sulfide (H2S) production. On the other hand, the tested isolates were positive for catalase, V.P, nitrate reduction test citrate utilizing, lysine decarboxylase urea hydrolysis test. Additionally, all *proteus isolates were* negative for oxidation indole, V.P and lysine decarboxylase. On the other hand, the tested isolates were positive for catalase, methyl red, hydrogen sulfide (H2S) production, citrate utilizing, nitrate reduction test and urea hydrolysis test. Finally, *pseudomonas* isolates were all negative for indole, V.P, methyl red, no hydrogen sulphide (H2S) production and urea hydrolysis test. On the other hand, the tested isolates were positive for oxidation test, catalase, citrate utilizing , nitrate reduction test and pigmentation.

Table (1): *prevalence of the isolated bacteria spp. on their specific media during 2021*

Isolated bacteria spp.	No of tested samples	Positive spp.	Prevalence
Salmonella	3844	328	8.5%
E. coli	2836	1770	62.4%
Klebsiella	2833	219	7.7%
Proteus	3836	68	1.7%
Pseudomonas	2840	70	2.4%

No.: Number of samples %**:** Percentages of positive samples

Table (2): *The prevalence of the isolated bacteria spp. On their specific media during 2022*

Isolated bacteria spp.	No of tested samples	Positive spp.	Prevalence
Salmonella	2589	252	9.70%
E. coli	1792	1252	69.80%
Klebsiella	1780	187	10.50%
Proteus	2541	0	0
Pseudomonas	1873	84	4.40%

No.: Number of samples **%:** Percentages of positive samples

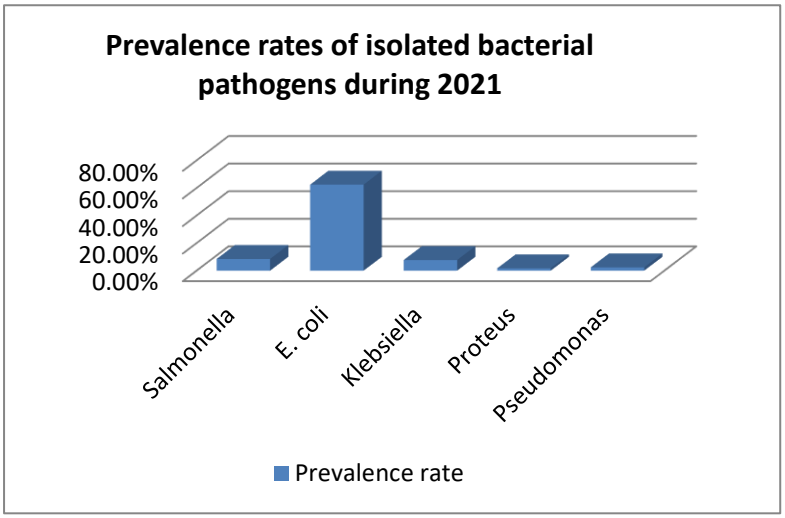

Fig. (1): *Prevalence of the isolated bacteria spp. and their prevalence on their specific media during 2021***.**

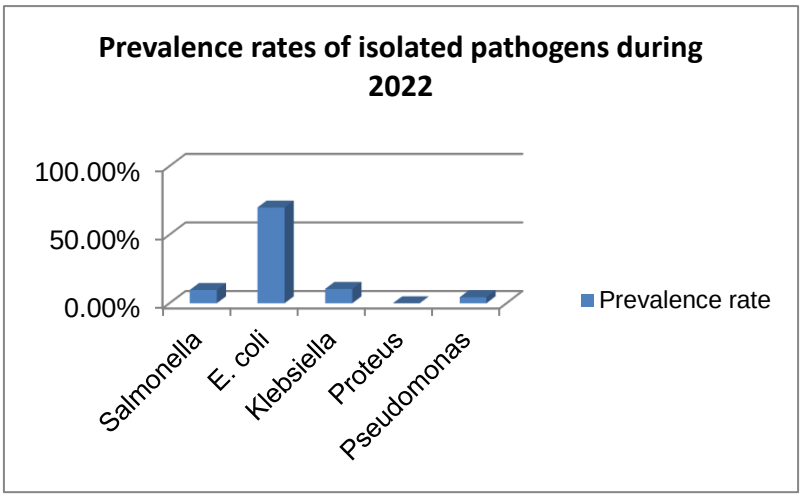

Fig. (2): *Prevalence of the isolated bacteria spp. and their prevalence on their specific media during 2022.*

Discussion:

In this study 580/6433 *Salmonella* isolates were recovered with 9% prevalence rate, this result agreed with **(*Duc et al., 2019*; *Naira et al., 2023*; *Muhammad et al., 2010* and *Zhao et al., 2020*)** who isolated *Salmonella* from broiler chickens with a prevalence of (7.9%, 8%, 9% and 11.2%). In addition to the results higher than that were obtained by **(*Ahmed et al., 2014*)** who isolated *Salmonella* from broiler chickens with a prevalence of (3.7 %). While the results disagreed with **(*Rania et al., 2024*)** who isolated *Salmonella* from broiler chickens with a prevalence of (62.5%).

In this study 3022/4628 of *E. coli* strains were recovered with 65.3% prevalence rate, this result nearby with **(*Rekaz et al., 2019*)** who isolated *E. coli* from broiler chickens with a prevalence of (53.4%). Lower results were obtained by **(*Jude et al., 2022*)** who isolated *E. coli* from broiler chickens with a prevalence of (20.56%). While the results disagreed with **(*Rania et al., 2024*)** who isolated *E. coli* from broiler chickens with a prevalence of (87.5%).

In this study 406/4613 *Klebsiella* isolates were recovered with 8.8% prevalence rate as in, this result nearby with **(*Jude et al., 2022*)** who isolated *Klebsiella* from broiler chickens with a prevalence of (9.9%) While the results disagreed with **(*Ejikeugwu et al., 2021*)** who isolated *Klebsiella* from broiler chickens with a prevalence of (33%).

In this study 68/6377 *Proteus* isolates were recovered with 1% prevalence rate this result was lower than **(*Li et al. 2022*)** who isolated *Proteus mirabilis* with 7.07% prevalence rate and the results lower than **(*Algammal et al., 2021*)** who isolated *Proteus* with prevalence rates of (14.6%) and the results dis agreed with **(*Nahar et al., 2014*)** who isolated *proteus* with prevalence rate of 38.6%.

In this study, the prevalence of *P. aeruginosa* in chickens was 3.2% agreed with (***Ashraf et al., 2016*** **and *Mohamed,* 2004)** who isolated *P. aeruginosa* from broiler chickens with a prevalence of (2.5% and 3.3%), respectively, and lower than that obtained **(*Jihan et al., 2020*)** who isolated *P. aeruginosa* from broiler chickens with a percentage of 69.57%

Conclusion:

This study revealed various bacterial pathogens recovered from freshly dead and diseased chicks that suffered from respiratory manifestations associated with diarrhea as *Salmonella*, *E. coli*, *Klebsiella*, *Proteus* and *Pseudomonas*, these pathogens cause significant financial losses for poultry owners and also have an indirect impact on public health.

Conflict of Interest

There are no disclosed conflicts of interest for the writers.

References:

Agnes, A.; Dave, L. and Carolee C. (2012): Review of antimicrobial therapy of selected bacterial diseases in broiler chickens in Canada. Can Vet J. 53(12): 1289–1300.

Ahmed, M.A.; Youssef,.F.M. and Abdel Rahman, A.G. (2014): Studies on the effect of thymol oil on *Salmonella* bacteria in broiler chicks. Assiut Vet. Med. J. 60 (140).

Algammal, A. M., Hashem, H. R., Alfifi, K. J., Hetta, H. F., Sheraba, N. S., Ramadan, H., & El-Tarabili, R. M. (2021): *atp* D gene sequencing multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR *Proteus mirabilis*. *Scientific reports*, 11(1), 9476.

Aly, M.M.; Khalil, S.A. and Metwaly, A. (2014): Isolation and molecular identification of *Klebsiella* microbe isolated from Chicks. AJVS.; 43(1): 97-103.

Ashraf A. Abdel-Tawab, Soad A. Nasef and Ola A. Ibrahim (2016): Bacteriological and Molecular Studies on Bacteria Causing Omphalitis in Chicks with Regard to Disinfectant Resistance. Global Veterinaria 17 (6): 539-545.

Basler, C; Nguyen, T.A.; Anderson, T.C.; Hancock, T. and Behravesh, C.B. (2016): Outbreaks of human *Salmonella* infections associated with live poultry, United States, 1990-2014. Emerg. Infect. Dis., 22(10): 1705-1711.

Collee, J.G.; Fraser, A. G.; Marmion, B.P. and simmons, A. (1996): Practical medical microbiology. 14th Ed., Chuechill, living stone.

Cruickshank, R.; Duguid, J. P.; Marmion, B. P. and Swain, R. H. A. (1975): Medical Microbiology. 12th Edn., Churchill, Livingstone, Edinburgh, UK. London and New York.

Duc, V.M.; Nakamoto, Y.; Fujiwara, A; Toyofuku, H. ; Takeshi O. and Takehisa C. (2019): Prevalence of *Salmonella* in broiler chickens in Kagoshima, Japan in 2009 to 2012 and the relationship between serovars changing and antimicrobial resistance. BMC Veterinary Research 15:108.

Ejikeugwu, C.; Okoro, N.; Morteza, S.; Hussein, O. and Al Dahmoshi, A. (2021): Metallo-β-lactamase and AmpC genes in *E. coli*, *K. pneumoniae*, and *Pseudomonas aeruginosa* isolates from abattoir and poultry origin in Nigeria. 18: 16.

Jihan, M.B.; Fawzy, R.El Saidy and Amal, A.A. (2020): Emergence of Multi-Drug Resistant Pseudomonas aeruginosa in Broiler Chicks. International Journal of Microbiology and Biotechnology; 5(2): 41-47.

Jude, F. L.; Innocent, M.A.; **Ousenu K. and Christopher B. T.** (2022): Patterns of Antibiotic Resistance in *Enterobacteriaceae*

Isolates from Broiler Chicken in the West Region of Cameroon: A Cross-Sectional Study. Canadian Journal of Infectious Diseases and Medical Microbiology, Volume 2022, Article ID 4180336, 18 pages.

Li, Z.; Peng, C.; Zhang, G.; Shen, Y.; Zhang, Y.; Liu, C.; Liu, M. and Wang, F. (2022): Prevalence and characteristics of multidrug resistant *Proteus mirabilis* from broiler farms in Shandong Province, China. Poultry Science. 101(4): 101-117.

Maram, M. T.; Nehal, M. N. and Reem, M. R. (2018): Molecular studies on some virulence factors of *pseudomonas aeruginosa* isolated from chickens as a biofilm forming bacteria. Assiut Vet. Med. J. Vol. 64 No. 159 October 2018, 43-51.

Mohamed, H. A. E. H. (2004): Some studies on *Pseudomonas* species in chicken embryos and broilers in Assiut Governorate. Assiut Univ Bull. Environ. Res., 7: 23-30.

Muhammad, M.; lawal U.M.; Abdul-G.A.; Aliyu U. M.; Samuel A. and Lisa, B. (2010): Prevalence of *Salmonella* associated with chick mortality at hatching and the susceptibility to antimicrobial agents. Veterinary Microbiology journal. 140(1-2):131-135.

Nahar, A.; Siddiquee, M.; Nahar, S.; Selim, K.; Anwar, K.; Ali, S. and Islam, S. (2014): Multidrug Resistant-*Proteus Mirabilis* Isolated from Chicken Droppings in Commercial Poultry Farms: Biosecurity Concern and Emerging Public Health Threat in Bangladesh. J Biosafety Health Educ. 2(2): 100-120.

Naira, M. AboSleima; Abdelazeem M. Algammalb; Wafaa, A. Abd El-Ghany; Nayera M. Alatfeehy (2023): *Salmonella* enterica Isolated from Diseased Broiler Chickens. SCVMJ, XXVIII (2):257-261.

Rania, s.; Moshira, E; Hanem, E. and Mahmoud, M.I. (2024): Prevalence, molecular characterization, and antimicrobial resistance among *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* strains isolated from Egyptian broiler chicken flocks with omphalitis. Open Vet J. 2024 Jan; 14(1):284-291. doi: 10.5455/OVJ.2024. V14.i1.25. Epub 2024 Jan 31.

Rekaz A. Ibrahim, Tillie L. Cryer, Shawkat Q. Lafi, Ehab-Abu Basha, Liam Good and Yaser H. Tarazi (2019): Identification of *Escherichia coli* from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. BMC Veterinary Research (2019) 15:159

Tantawy, M., Amer, H. A., El-Khyate, F. F., & El-Abasy, M. (2018): *Klebsiella Pneumoniae* infection in broiler chickens. Kafr El-Sheikh Veterinary Medical Journal, 16(1), 17-42.

Ulmer Franco, A.M. (2011): Yolk Sac Infections in Broiler Chicks,

Ph.D. thesis, University of Alberta Edmonton, Alberta, pp: 1-197.

Zhao, X.; Hu, M.; Zhang,Q.; Zhang,C.Y. ; Li, L.; Qi,J.; Luo, Y.; Dong Zhou, D. and Liu, Y. (2020): Characterization of integrons and antimicrobial resistance in *Salmonella* from broilers in Shandong, China. Poultry Science 99:7046–7054.

معدل انتشار البكتيريا المختلفة المعزولة من كتاكيت التسمين التي تعاني من أعراض تنفسية مصحوبة بالإسهال

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الملخص العربي

يعد الجهاز التنفسي بمثابة الموقع الرئيسي لعدوى العديد من مسببات الأمراض التي تسبب الأمراض المزمنة في الطيور، كما أن مشاكل الجهاز التنفسي هي السبب الرئيسي للخسائر المالية في صناعة الدواجن. هدفت هذه الدراسة إلى عزل مسببات الأمراض البكتيرية المختلفة التي تصيب أفراخ الدجاج اللاحم. تم جمع العينات من الكتاكيت النافقة والمريضة والتي تعاني من أعراض تنفسية مرتبطة بالإسهال. تم اختبار هذه العينات (عددها = 6433 و 4628 و 4613 و 6377 و4713) لعزل السالمونيلا، الميكروب القولوني، الكلبسيلا،البروتيوس و السودوموناس، على التوالي. تم جمع العينات من أفراخ اللاحم بعمر يوم إلى 35 يوم من الواحات، ريجوا (شركة القاهرة 3 أ) والمزارع الخارجية، مصر. وقد كانت نسب العزل للميكروبات كالتالي 9%، 65.3%، 8.8%، 1% و3.2% للسالمونيلا، والميكروب القولوني، والكلبسيلا، والبروتيوس، والسودوموناس على التوالي. وتم التعرف على جميع مسببات الأمراض البكتيرية المعزولة كيميائيا باستخدام اختبار الإندول، اختبار الميثيل الأحمر (MR)، اختبار فوجيس-بروسكاور (VP)، اختبار استخدام السترات، اختبار الكاتالاز، اختبار الأوكسيديز، اختبار إنتاج كبريتيد الهيدروجين، اختبار إنتاج اليورياز، تخمير السكر واختبار ليسين ديكاربوكسيلازو ختبار اختزال النترات، أجار الحديد الثلاثي السكر.