MedCrave
Step into the World of Research
International Journal of Molecular Biology Open Access

Research Article
Open Access
CrossMark

Molecular characterisation of pathogens isolated from egg samples

Abstract

ceA on the biochemical analysis of this study, which is similar to a battery of biochemical tests including methyl red, Voges-Proskauer, indole and citrate, the organisms were determined to be *Klebsiella*. In the present study, the highest resistance rate was observed for inoflavuxin (50%) and ceftazidimine (80%), which is similar to the highest resistance rate for cefotaxime (85%) and ceftazidime (68%), with the MIC ranging from 32 to 256 mg/l. The *Pseudomonas* and *Klebsiella* plasmid isolation in the present study was found to have a molecular weight of 2.6 kb and 2.7 kb, which is similar to most isolates harboring multiple plasmids ranging in size from about 2 kb to over 150 kb included. Seventy-nine isolates contained plasmids larger than 50 kb, and some contained multiple large plasmids. In the present study, the molecular weight of *Pseudomonas* and *Klebsiella* was found to be 356 base pairs and 359 base pairs, respectively, which is similar to that of the 85 clinical isolates of *Klebsiella* pneumonia screened by PCR amplification of the antiseptic resistance genes qacE1 and ceA.

Keywords: *pseudomonas, klebsiella,* pathogens, molecular characterization

Volume 7 Issue 1 - 2024

P Sagadevan,[1] Jenifer Christina,[1] M Raghunath,[1] M Nagarajan,[1] S Premalatha,[1] R Preeethi,[1] Janarthanan P[2]

[1]PG Department of Biotechnology, KSG college of Arts and Science Coimbatore, Tamilnadu, India
[2]Kovai Medical Center and Hospital, Coimbatore, Tamilnadu, India

Correspondence: Dr P Sagadevan, Assistant Professor, PG Department of Biotechnology, KSG college of Arts and Science, Coimbatore, Tamilnadu, India,
Email sagadevan.biotec@gmail.com

Received: December 18, 2023 | **Published:** January 10, 2024

Introduction

Foodborne illnesses represent major public health problems and are a major cause of diarrheal diseases affecting all developed and developing countries.[1] Eggs have a natural defence system against contaminating microorganisms such as the epidermis, hard calcium shell and shell membrane.[2] Egg whites contain several egg white proteins that have antimicrobial properties, including lysosome. Mucoid is another proteins that inhibits the ability of bacteria to utilize proteins in proteins. Furthermore, a protein pH of around 9 to 10 and protein viscosity do not support the growth of microorganisms.[3] The egg may be contaminated both on the shell and on its contents by various microorganisms with a wide range of pathogens such as Campylobacter jejune, Listeria monocytogenes, Escherichia coli, Yersinia enterocolitis and especially Salmonella.[4] Contamination of eggshells increases the risk of contamination of the egg contents through penetration.[5] Bacterial infection can occur in all three main parts of the egg (yolk, albumen and egg membrane/shell).[6] In the present study, a survey was conducted to determine the aerobic bacterial load and pathogens present on and in eggshells. Pathogens were also tested for susceptibility testing. Eggs have been used as food by humans since ancient times.[7] The yolk and whole egg store significant amounts of protein and choline[8] and are often used in cooking. Because of their protein content, the U.S. Department of Agriculture classifies eggs as meat in the food pyramid. Eggs provide a good combination of nutrients, providing all essential human amino acids as well as several vitamins and minerals.[9]

Freshly eggs are considered a delicacy in many parts of the world, including Europe and North America. In Japanese cuisine, they are sometimes used raw or cooked like tam ago in sushi and are often found in bento lunches.[10] Nowadays, it is not uncommon in Nigeria to see people living on quail eggs. People who eat eggs do so for their supposed medicinal properties and the nutrients they contain.[11] Foodborne illnesses caused by microorganisms represent a significant and growing public health problem.[12] Contamination of eggs and egg products by microorganisms can affect egg quality and lead to egg spoilage and transmission of pathogens. This can lead to foodborne infections or poisoning in consumers. Today, eggs remain a staple food consumed by people around the world. They are consumed worldwide in the form of cookies, stews and drinks and are considered a highly nutritious and inexpensive source of protein.[13] Although eggs are considered complete food for growth and maintenance, research suggests that eggs are often contaminated by microorganisms.[14] Freshly laid eggs are generally free of organisms. However, when eggs are exposed to environmental conditions such as soil, feces, and dirty nest material, they become contaminated with various types of microorganisms.[15] In addition, these microorganisms can contaminate egg contents by entering or excreting through the pores of the shell[16] and transversally.[17] Predisposing factors such as ambient temperature and humidity influence bacterial invasion, increasing infection and spoilage.[18]

Pathogenic microorganisms have been isolated from the surface of chicken eggshells and their contents. These include Listeria monocytogenes, Yersinia enterocolitis, Escherichia coli O157:H7, Salmonella and Campylobacter SPP.[19] Other pathogens include the fungal organisms Aspergillus, Penicillin etc.[20] Antitoxins, produced by some species of fungi, contaminate a wide range of food and agricultural products. These mycotoxins pose a serious food safety problem in many countries, particularly in tropical and subtropical regions, where temperature and humidity are optimal for mold growth and toxin production. The possibility of transferring these toxic residues to edible eggs poses a potential risk to human health.[21] A chicken egg is very rich in nutrients and is therefore one of the most valuable and perfect foods. Eggs can fully satisfy the needs of organisms, including humans, for all nutrients necessary for their development and vital functions. At the same time, many nutrients contained in eggs create an excellent environment for the development of bacterial microflora, including pathogenic bacteria. The fact that eggs can be contaminated or infected horizontally (through the shell) or vertically (transversally) makes them a potential source of pathogens is currently considered an urgent threat to human health due to the emergence of multidrug-resistant strains associated with nosocomial epidemics and strains associated with severe nosocomial infections. Pneumonia is ubiquitous in the environment and can colonize and infect both plants and animals. However, little is known

about the population structure of pneumonia, making it difficult to detect or understand the emergence; clinically important clones within this genetically extremely diverse species. Here, we present a detailed genomic picture of pneumonia based on whole-genome sequencing of more than 300 human and animal isolates from four continents. The data provide broad genomic support for the division of pneumonia into three distinct species, KPI pneumonia APII (K.quasi pneumonia) and Kiwi (K. Variicola). Furthermore, in the case of pneumonia (KPI), the disease most commonly associated with human infections, we demonstrate the existence of more than 150 deeply branched lineages, including numerous multidrug-resistant or clones. We show that pneumonia has a large additional genome comprising nearly 30,000 protein-coding genes, including a number of virulence functions that are significantly associated with community-acquired invasive diseases in humans. In our data set ;, antimicrobial resistance genes were commonly found in isolates from human carriers and nosocomial infections, which generally lacked genes associated with invasive disease. The convergence of virulence and resistance genes could potentially lead to the emergence of incurable invasive pneumonia infections; our data provides a genome-wide picture that can be used to track the emergence.[17] (gram-negative bacterium pneumonia is the leading cause of healthcare-associated infections (HA) and neonatal sepsis worldwide.[22] Pneumonia, generally considered an opportunistic pathogen, can spread asymptomatically in the gastrointestinal tract, skin, nose and throat of healthy people,[23] but can also cause a range of infections in hospitalized patients, very often pneumonia and wounds. And soft tissue or urinary tract infections. pneumonia infections represent a particular problem in infants, the elderly and immunocompromised individuals,[24] but also cause a significant number of serious infections in the community, including pyogenic liver abscesses, pneumonia and cerebrospinal meningitis.[25] Virulence factors thought to be associated with invasive CA infections include various siderophores, specific stereotypes of polysaccharide capsules, and MPA genes.

Materials and methods

Collection of samples

50 Eggs were collected from different farms to isolate the bacteria present in its shell and in its contents. The eggs were purchased less than four days old from the date of production. All the samples were processed within four hour of its collection.

Isolation and identification of organism

Nutrient broth was prepared for 15 ml and dispersed into three test tubes and named as A1, A2 and A3respectively.With the help of tooth pick egg swapping was done and kept in incubation at 37°C for 24 hours. After incubation, the samples (A1, A2 and A3) were streaked on MacConkey agar and urea base agar using sterile wire loop. After streaking the plates were incubated at 37℃ for 24 hours.

Biochemical test

In every two plates A1, A2 had shown micro-organisms. Nutrient broth 10 ml was prepared and sterilized along with two test tubes. After sterilization nutrient broth was shared into two test tubes. Micro-organism on A1 was shared into one test tube and micro-organism on A2 was shared into another test tube and kept in incubator for overnight.

Citrate utilization test

By using sterile technique method inoculated each bacterium into its appropriately labeled tube by means of stab and streak inoculation. All cultures were incubated for 24 to 48 hours at 37°C and were observed.

Citrimide agar

Citrimide agar of 15ml was prepared and poured in petri plate. After incubation A1 micro-organism was taken and streaked in citrimide agar plate and kept in incubation for overnight.

Indole test

0.5ml of 24 hours to 48 hours peptone water cultures of A2 micro-organism were taken in a small test tube. 0.2ml of Kovac`s reagent added to the peptone water and shaked Allow it to stand for few minutes and results were observed.

Methyl red test

0.5ml of broth cultures of A2 micro-organisms was taken in a small test tube. Five drops of 0.04%solution of methyl red was directly added to the broth culture and mixed well. Change in the colour of medium was observed.

Voges-Prokauer test

1ml of broth cultures of A2 micro-organism was taken in a small test tube. First added 40% KOH and followed by addition of 0.6ml of a 5% solution of α naphthol in ethanol to the broth culture and shaked gently. Change in the colour of medium within 2-5 minutes was observed.

Minimum inhibitory concentration analysis

1.68g of nutrient agar was added to 60ml of distilled water in an Erlenmeyer flask. The nutrient agar with four petri plates was autoclaved at 121°C for 15 minutes. Under sterile condition the nutrient agar was poured to four plates. After solidification of two petriplates were swabbed with *Klebsiella sp* and other two petri plates were swabbed with *Pseudomonas sp.* One Ofloxacin hicomb was placed in *Pseudomonas* plate and another Ofloxacin hicomb was placed in *Klebsiella* plate and samewise ceftazidime hicombs were placed in *Pseudomonas* and *Klebsiella* plates. Those plates were placed in incubator for overnight. Measurement of bacterial growth by optical density was carried out. LB broth was prepared in 10 ml and shared in two test tubes. *Pseudomonas* sp (100µl) was shared in one test tube and *Klebsiella* sp (100µl) was shared into another test tube. Chloramphenicol (10µl) was shared into both the test tubes and kept in incubator for 24 hours.

Plasmid isolation

Pseudomonas and *Klebsiella* of standard culture samples were taken in eppendorf tube. Centrifugation was made for 3 times with 8000 rpm for 16 mins. After centrifugation pellets were collected and supernatants were discarded. Then solution A 100 µl with lysozyme of 20 µl were added to the pellets. Then placed in room temperature for 30 minutes. Then solution B 200 µl was added. Invert the tube and incubate on ice for 10 min. After incubation solution C of 150 µl was added. Then vortex and kept in ice bath for 30 minutes. After that supernatant was collected and the pellets were discarded. Centrifuge was done at 10000 rpm for 10 minutes. After that 2 µl of isopropanol was added. Centrifuge the tubes at 1000 rpm for 10 minutes and the pellet was collected. To this 3 M sodium acetate 40 µl and 100 µl of absolute ethanol was added and kept in deep freeze for 30 minutes. Tubes were taken from deep freeze centrifugation were done for 5000rpm for 5 minutes. After centrifugation pellet was collected. The pellet was mixed with 700µl of 70% ethanol. Centrifuge was

done at 5000rpm for 5 minutes. And then pellet was collected and supernatants were discarded. 50µl 1xTE buffer was added with the above pellet.

Plasmid was collected and isolated plasmid was coated with dye and electrophoresis was carried out. The bands were observed under UV-Trans illuminator.

PCR

- Template plasmid 2µl
- primer 1 µl
- PCR master mix (1µl)
- PCR buffer (5µl)
- Distilled water (3µl) (Table 1)

Table 1 PCR Conditions

Initial denaturation	94°C	30 seconds
Denaturation	94°C	1 minute
Annealing	54°C	15 seconds
Extension	72°C	30 seconds
Final extension	72°C	3 minutes
No.of cycle	20 cycles	

After taken from PCR and in electrophoresis were carried out. The bands were observed under UV Trans-illuminator

RFLP-Restriction fragment length polymorphism

- DNA - 2 µl
- Restriction enzyme - 2µl (restriction enzyme were EcoRl, BamHl and Hindlll)
- 10X Restriction mixture-(2µl)
- Sterile water -(2µl)
- The samples were incubate at 37°C for 3-24 hour incubation and run in 1% agarose electrophoresis.

Cell wall protein

LB broth was prepared for 10ml and shared in two test tubes. Then *Pseudomonas sp* (100 µl) and *Klebsiella sp* (100 µl) were added in each tube and kept in incubation for overnight. After incubation period of 2ml culture are transferred to eppendorf tube. Centrifuge the tubes at 18000 rpm for 12 minutes. Collect the pellet and discard the supernatant after centrifugation. Add lysis buffer 500 µl and10ml of lysozyme and 10 ml of 15%sucrose to the above pellet. Incubate in ice for 60 minutes. Centrifuge the tubes at 4000 rpm at 12 minutes. After centrifugation collect the supernatant and discard the pellet. Then again centrifuge at 10,000 rpm for 20 minutes. After centrifugation add 10%SDS of 500 µl. Keep the tubes on deep freeze for a day. The samples were centrifuge at 5000 rpm for 10 minutes. Collect the supernatant and discard the pellet. The supernatant were collected after centrifugation and kept in dialysis membrane for overnight at room temperature.After the incubation transfer supernatant from dialysis membrane to eppendorf tube. Take 60 µl in eppendorf tube and transfer in another eppendorf tube with the addition of SDS. The apparatus were cleaned and the reagents were prepared. The casting frames were set on the casting stand and glass plates were placed on the stands. Agarose gel was poured in between the stands and was allowed to solidify. Once the gel solidifies, the separating gel was poured between the glass plates. The separating gel was allowed to gelate. Then stacking gel was poured and the well-forming comb was inserted and let to gelate. Meanwhile the samples were stained and were kept in the water bath for 10 minutes at 90^0C.

After solidification, the glass plates were removed from the frame and were placed in the buffer tank. The buffer tank was filled with the running buffer and the samples were loaded onto the well. A protein marker was added in one of wells. The maker used here was medium range standard protein. The top of the dam was covered and was connected with the anode and cathode. The volt was set and electrophoresis of the sample in the gel was performed. Once the maker and the sample reach 3/4th of the gel, the voltage was stopped and was removed from the chamber. The gel was then stained with CBB (coomassie brilliant blue) dye. Staining was followed by de-staining process. After 24-48 hours the gel was subjected to de-staining. The gel was analysed for the protein.

Remedial measures

1.14g of Mullon-Hinton agar was added to 30 ml of distilled water in an Erlenmeyer flask.The Mullon-Hinton agar along with two petri plates was autoclaved at 121 C° for 15 minutes. Under sterile condition Mullon-Hinton agar was poured on to two petri plates. After solidification *Pseudomonas* and *Klebsiella* was cotton swabbed on separate two plates. With the help of Gel puncher four wells were made in both the plates. Then Zinc nano particle (20 µl), silver nano particle (20 µl), gold nano particle (20µl) and plant extract (20 µl) was poured on to the wells and kept in incubator for overnight.

Results and discussion

Identification of organisms

Identification and characterization of microorganisms were carried out in MacConkey agar and urea base agar (Figure 1).

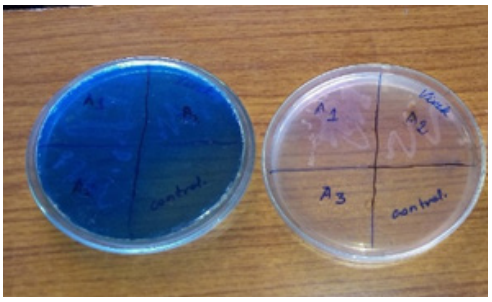

Figure 1 A1 and A2 show the growth of microorganisms.

Biochemical characterization

IMVIC test

Following Table 2 shows the result of IMVIC test and shows the presence of A2 strain as *Klebsiella* organisms.

Table 2 Results of IMVIC test

Test	Color	Identification
Indole	Yellow ring appears	Negative
Methyl red	Yellow colour appears	Negative
Voges- Proskauer	Pink colour appears	Positive
Citrate Utilization	Deep blue colour appears	Positive

A1 organism was identified as *Pseudomonas* by the colonies grown on citrimide agar. It was observed on Figure 2.

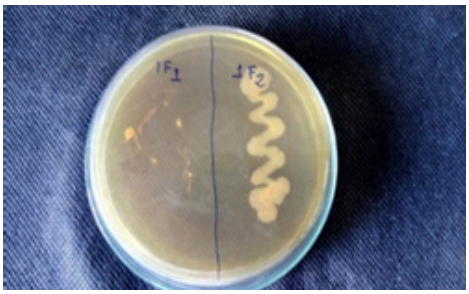

Figure 2 Citrimide agar.

MIC

An antifungal Inhibitory Concentration is the lowest concentration of an antimicrobial (like an antifungal, antibiotic or bacteriostatic) drug that will inhibit the visible growth of a microorganism after overnight incubation Figure 3 &4. *Pseudomonas* forms zone only in ofloxacin and *Klebsiella* forms zone in both the hicombs. It was observed on Table 3& 4. The molecular weight for *Pseudomonas* and *Klebsiella* was found to be 2.6, and 2.7 kbs. It was observed from Figure 5.

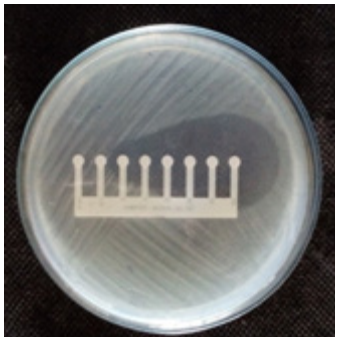

Figure 3a Ofloxacin *(Pseudomonas)*.

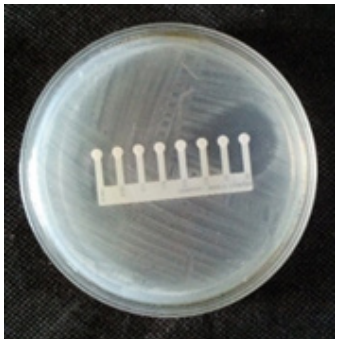

Figure 3b Ceftazidime (*Pseudomonas)*.

Figure 4a Ofloxacin (*Klebsiella*).

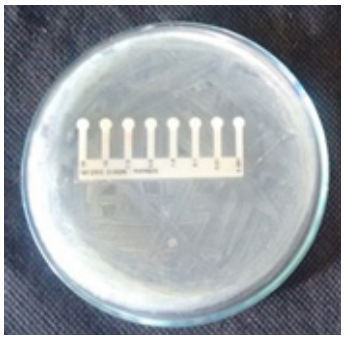

Figure 4b Ceftazidime (*Klebsiella*).

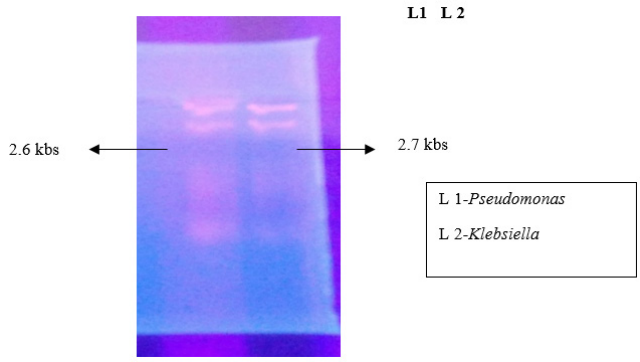

Figure 5 Plasmid isolation for *Pseudomonas* and *Klebsiella.*

Table 3 MIC analysis of ofloxacin hicomb

CONC.(µg)	*Pseudomonas*	*Klebsiella*
0.01	Resistant	Resistant
0.1	Resistant	Resistant
2	Resistant	Resistant
4	Resistant	Resistant
8	Resistant	Intermediate
16	Intermediate	Intermediate
32	Intermediate	Sensitive
64	Intermediate	Sensitive

Table 4 Mic analysis of ceftazidime hicomb

CONC.(µg)	*Pseudomonas*	*Klebsiella*
0.001	Resistant	Resistant
0.01	Resistant	Resistant
0.1	Resistant	Resistant
1.0	Resistant	Resistant
3.0	Resistant	Resistant
7.5	Resistant	Resistant
15	Resistant	Resistant
30	Resistant	Resistant

PCR analysis

The polymerase chain reaction is a technique used in molecular biology to amplify a single copy or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence The molecular weight was found to be 356 basepair and 359 basepair for *Pseudomonas* and *Klebsiella* sp. It was observed from Figure 6.

Citation: Sagadevan P, Christina J, Raghunath M, et al. Molecular characterisation of pathogens isolated from egg samples. *Int J Mol Biol Open Access.* 2024;7(1):1–6. DOI: 10.15406/ijmboa.2024.07.00157

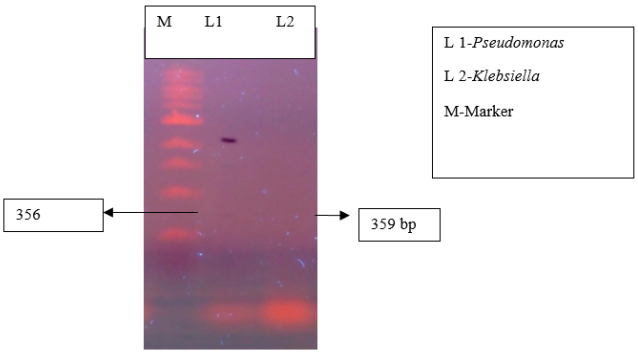

Figure 6 PCR for *pseudomonas* and *Klebsiella.*

RFLP

Restriction fragment length polymorphism or RFLP is a technique that exploits variations in homologous DNA sequences. In RFLP analysis, the DNA sample is broken into pieces and (digested) by restriction enzymes and the resulting restriction enzymes and the resulting restriction fragments are separated according to their length by gel electrophoresis Figure 7. The DNA was subjected to restriction analysis and four cuts were observed. The molecular weight of *Pseudomonas* and *Klebsiella* was found to be 2.1kbs to 2.4kbs and1.5kbs to 1.8 kbs respectively. It was noted from Cell wall protein of *Pseudomonas* was found to be 72kDa and *Klebsiella* was found to be 68 kDa. It was observed from Figure 8.

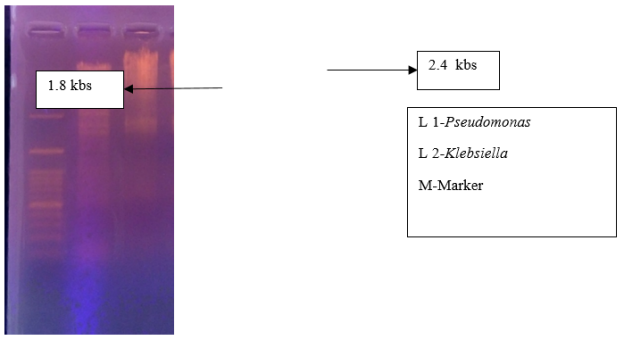

Figure 7 RFLP for *Pseudomonas* and *Klebsiella* sp.

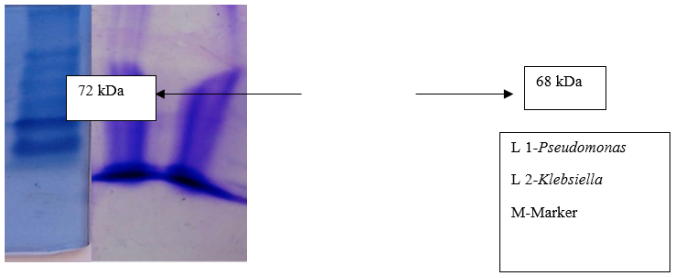

Figure 8 Cell wall protein for *Pseudomonas* and *Klebsiella.*

Conclusion

Pseudomonas this study, the MIC ranged from 32-256 mg/l and the highest resistance rates were found to be 50% (*Pseudomonas*) and 80% (*Klebsiella*) pneumonia. The majority of isolates 79 had multiple plasmid sizes ranging from about 2.5 kb to over 2.7kb, with 75% of isolates containing plasmids greater than 50kb and some isolates containing multiple large Plasmids. The molecular weight of the isolates in this study was 2.6kb (2.7kb) and 359 bp (359B), similar to the molecular weight found in the 85 Clinical isolates. Antiseptic Resistance Genes Screened by PCR Amplifications.

Acknowledgments

None.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Abuzaid A, Hamouda A, Amyes SGB. *Klebsiella pneumonia*suspectibility to biocides and its association with *cepA, qacEΔ1* and *qacE*efflux pump genes and antibiotic resistance. *J Hosp Infect*. 2012;81(2):87–91.
2. Adesiyun A, Offiah N, Seepersadsingh N, et al. Microbial health risk posed by table eggs in Trinidad. *Epidemiol Infect.* 2005;133(6):1049–1056.
3. *How to buy eggs*. Agricultural Marketing Service; USDA: 2011.
4. Akbar A, Anal KA. Zinc oxide nanoparticles loaded active packaging a challenge study against *Salmonella typhimurium*and *Staphylococcus aureus*in ready- to-eat poultry meat. *Food Control*. 2014;38:88–95.
5. Azadpur M, Nowroozi J, Goudarzi, et al. Presence of *qacEΔ1* and *cepA* and susceptibility to a hospital biocide in clinical isolation of *Klebsiella pneumonia* in Iran . *Trop Biomed.* 2015;32(1):109–115.
6. Backpacker Ben. Eat cheap, top 7 street food around the world. 2010.
7. Bagley ST. Habitat association of *Klebsiella* species. *Infect Control*. 1985;6(2):52–58.
8. Bialek DS, Alexis C, Florent A, et al. Genomic definition of hypervirulent and multi drug resistant *Klebsiella pneumoniae* clonal groups. *Emerg Infect Dis*. 2014;20(11):1812–1820.
9. Blumenthal D. *From the chicken to the egg*. FDA. pp 7–10; 1990.
10. Breed RS, Murray EGD, Hitchens, AP. *Bergey's manual of determinative bacteriology*. 6th ed. 1948.
11. Brisse S, Grimont F, Grimont P. *The Prokaryotes A Handbook on the Biology of Bacteria*. 3rd edn, Dworkin M, Falkow S, Rosenberg E, et al. New York; Springer: Proteobacteria: Gamma Subclass. 2006.
12. Brisse S, Passet V, Grimont PA. Description of *Klebsiella quasipneumoniae* sp. novel isolated from human infections, with two sub-species, *Klebsiella quasipneumoniae* spp. quasipneumoniae subsp. nov, and *Klebsiella quasipneumoniae* subsp. similipneumoniae subsp. nov., and demonstration that Klebsiella singaporensis is a junior heterotypic synonym of *Klebsiella variicola*. *Int J Syst Evol Microbiol*. 2014;64:3146–3152.
13. Brisse S, Verhoef J. Phylogenetic diversity of *Klebsiella pneumoniae* and Klebsiella oxytoca clinical isolates revealed by randomly amplified polymorphic DNA, gyrA and parC genes sequencing and automated ribotyping. *Int J Syst Evol Microbiol*. 2001;51:915–924.
14. Brisse S. Virulent clones of *Klebsiella pneumoniae*: Identification and evolutionary scenario based on genomic and phenotypic characterization. *PLoS ONE*. 2009;4(3):e4982.
15. Broberg CA, Palacios M, Miller VL. *Klebsiella:* A long way to go towards understanding this enigmatic jet-setter. *F1000Prime Rep*. 2014;6:64–76.
16. Bruce J, Drysdale EM. Trans shell transmission. *Microbiology of the avian egg*. London: Chapman and Hall, 1994;63–91.
17. Buchanan RE, Gibbons NE, eds. *Bergeys manual of determinative Bacteriology*. 8th edn. Baltimore: The Willliams and Wilkins, 1974.
18. Casey BB, Ian TW, Robert VT. Emerging food borne pathogens and problems; expanding prevention efforts before slaughter or harvest. *Improving food safety through one health approach. Work shop summary*. US: National academies press; pp 1–418, 2012.

19. Centers for disease control and prevention antibiotic resistance Threats in the United States. 2013.

20. Chen L, Barun M, Kalyan D, et al. Carbapenemase-producing *Klebsiella pneumoniae*: molecular and genetic decoding. *Trends Microbiol.* 2014;22(12):686–696.

21. Chen L, Mathema B, Pitout JD, et al. Epidemic *Klebsiella pneumonia* ST258 is a hybrid strain. *mBio*. 2014;5(3):01355.

22. De Reu K, W Messens, M Heyndricks, e al. Bacterial contamination of table eggs and the influence of housing systems. *World's Poult Sci J*. 2008;64(1):5–19.

23. Deleo FR. Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*. *Proc Natl Acad Sci USA*. 2014;111(13):4988–4993.

24. Dhiaa J Hamzah. Isolation of some bacterial contamination of egg shell and yolk in najaf governante. *Journal for Veterinary Medical Sciences*. 2014;5(2):119–133.

25. Euzeby JP. List of bacterial names with standing in nomenclature: a folder available on the Internet. *Int J Syst Bacteriol.* 1997;47(2):590–592.

26. Falade AG, Ayede AI. Epidemiology, aetiology and management of childhood acute community-acquired pneumonia in developing countries—a review. *Afr J Med Med Sci*. 2011;40(4):293–308.

Citation: Sagadevan P, Christina J, Raghunath M, et al. Molecular characterisation of pathogens isolated from egg samples. *Int J Mol Biol Open Access*. 2024;7(1):1–6. DOI: 10.15406/ijmboa.2024.07.00157