Available online on 15.06.2023 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

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Research Article

Molecular Characterisation of Multidrug Resistant Pathogen Isolated from Egg and its Control Measures

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Article Info:

Article History:

Received 21 March 2023
Reviewed 09 May 2023
Accepted 26 May 2023
Published 15 June 2023

Cite this article as:

Rajendran S, Selvakumar P, Kaladharan S, James J, Molecular Characterisation of Multidrug Resistant Pathogen Isolated from Egg and its Control Measures, Journal of Drug Delivery and Therapeutics. 2023; 13(6):45-50

DOI: http://dx.doi.org/10.22270/jddt.v13i6.5852

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Abstract

Multidrug resistance is a condition enabling a disease causing microorganism to resist distinct drugs or chemical of wide variety of structure and function targeted at eradicating the bacteria. Microorganisms that display multidrug resistant can be pathogenic cells. In this study, table eggs are used commonly, because it considered the most nutritious economical source of protein that can be a part of healthy diet. However, egg shell carries bacteria which cause illness; even unbroken clean fresh shell egg may also contain harmful bacteria. In egg shell presence of *Escherichia coli* and *Staphylococcus aureus were found*. Out of total examined sample, no Salmonella was detected. Several disease occurred in poultry are caused by Staphylococcus sp. such as *Staphylococcus aureus*. It produces Enterotoxins which create food poisoning in consumers. Eggs are the potential source of transmitting antibiotics. Resistant Staphylococcus strain to human causing food-borne infection through Staphylococcus strains can potentially be harmful to humans. Methicillin-resistant *Staphylococcus aureus* (MRSA) is considered as one of the important bacterium. As a result, Multidrug resistance (MDR) in the bacteria may develop. Nowadays poultry farm increase day by day in Tamilnadu, there is a chance of transmitting the MRSA to Human through egg consumption. This present study was conducted to isolate the bacterial species and the characterization of *Staphylococcus aureus* along with remedial measure on egg Shell surface.

Keywords: Multidrug resistant, Pathogenic, Egg shell, Antibiotics, Human welfare

INTRODUCTION

Multiple drug resistance or multi drug resistance is a condition enabling a disease causing organism to resist distinct drugs or chemical of a wide variety of structure and function targeted at eradicating the organism, organisms that display resistance can be pathologic cells, including bacterial and neoplastic cell [1,2]. Multidrug resistant organisms (MDROs) are defined as microorganisms that are resistant to one or more classes of antimicrobial agent [3,4]. Multi drug resistance in bacteria occurs by the accumulation on resistance plasmid or transposes of gene with each coding for resistance to a specific agent or by the action of multidrug resistance.Multidrug resistance in bacteria may be generated by one of two mechanisms [5 6]. The discovery of penicillin in 1928 was followed by the discovery and commercial production of many other antibiotics. Any infection disease is curable by antibiotics therapy and their use had a profound impact on the life of bacteria on earth.More strain of pathogens have become resistant to many antibiotic and chemotherapeutic agents, the phenomenon is multidrug resistance. Some strains have been resistant to practically all of the commonly available agents [7].

A notorious case is the Methicillin-resistant *Staphylococcus aureus* (MRSA), such strain are also resistant to disinfectants and MRSA can act as a major source of hospital acquired infection. An even more serious threat may be the emergence of gram negative pathogens that are resistant to essentially the entire available agent. Thus, there are newly developed agent that are active against vancomycin resistant MRSA, such as linezolid. The emergence of "pan–resistant"gram negative strains, notable those belonging to *Pseudomonas aeruginosa* and *Acinetobacter baumanni*, occurred more recently, after most major pharmaceutical companies stopped the development of new antibacterial agents.

There is almost not agent that could be used against this strain, in an outer membrane barrier of low permeability and an array of efficient multidrug combined with Multitudes of specific resistance mechanism [8-11].

Figure 1: ***Andrographis paniculata***

Andrographis paniculata is a bitter tasting annual plant prevalent in much of Asia. It is often used in combination with other herbs in traditional medicine to treat infectious diseases and associated fevers [12-14]. It is also used in folk medicine to treat snakebites. Andrographis is promoted in supplemental form for cancer prevention or treatment, and to counter chemotherapy toxicity in humans. Formulations containing standardized extracts of Andrographis are also marketed for colds and flu [15-16].

The bioactive compounds present in *A. paniculata* enhance the antimicrobial property[1]. Ethanol extract of *A. paniculata* has been reported to possess antibacterial activity against pathogenic and non-pathogenic bacteria including *E. coli*, *S. typhimurium*, *P. vulgaris*, *B. subtilis* and *S. aureus* .

MATERIALS AND METHODS

Preparation of egg isolates

Collection of egg samples from private poultry farm located in Coimbatore, Tamilnadu, and India. Wash all the eggs with normal potable water and inoculate the water sample into nutrient broth .

Specific media or differential media

Differential media used to isolate and identity microorganisms. It allows the characterization of several microorganisms based on the growth patterns. The biochemical characteristics of microorganisms are used in the differentiation and identification of the microorganisms among other microorganisms. This media use indicators and allow several closely related microorganisms to grow in the medium.

Antibiotic sensitivity test

Disc diffusion method is a widely used technique for determiningthe susceptibilityof bacteria to specific antibiotic. The zone inhibition formed can be used forinterpretingthe result such that susceptible and resistant strain of bacteria can beidentified. After the period of inhibition, the pathogenic organism grew well inMHA medium except in the region of the zone of inhibition. The zone of inhibitionformed was measured for each disc. The zone of inhibition formed was comparedwith zone interpretative standard values.

Antibiotic Disc Name

Table 1: List of Antibiotics used in this study

S. No	Code of the Drug	Name of the Drug
1.	CZ 30	Cefazolin
2.	MET5	Methicillin
3.	P10	Penicillin-G
4.	GAT 5	Gatifloxacin
5.	AMS 30/15	Amoxycillin / sulbactam
6.	CAZ 10	Ceftazidime
7.	C 30	Chloramphenicol
8.	AMP 10	Ampicillin

ELISA Test

Prepare 10 ml LB broth. 25 g in 10 ml distilled water. After sterilization use 96 well plates. In 96 well plate, first 3 columns and rows were used. In first row 50µl freshly prepared LB brothwereadded in 3 columns, second row first 50µl add freshly prepared broth and 50µlcultural media in 3 columns. In third row first add 50µl freshly prepared LB Broth, 50µlcultural media, and drugs added in different concentration each column 10µl, 20µl, 30µl, concentration.Finally, ELISA plates were incubatedfor24hrs.

Preparation of Plant Extract

Take 1 g of *Andrographis paniculata* leaf. Grinded the leaf will with a morter and pestle and little amount of water. Replace

the plant extract to conical flask and place the shaker for 1 hr at 60-70rpm. Then filter the plant extract. Apply the filtered plant extract to the egg shell.

RESULTS AND DISCUSSION:

Antibiotic sensitivity test

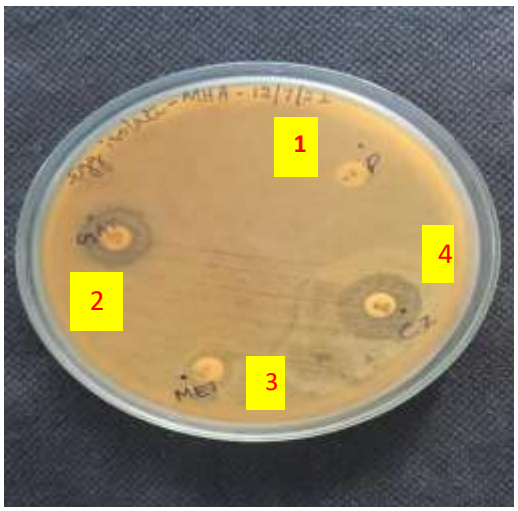

Figure 2: Antibiotics sensitivity test for egg isolate.1. P-Penicillin-G 2. 3. MET5 –Methicillin 4. CZ 30 – cefazolin

Minimum inhibitory concentration

Table 2: Measurement of Zone Formation

S.No	Disc name	Zone
1.	GAT	2 mm
2.	P	Nil
3.	CZ	4 mm
4.	MET	0.1 mm
5.	CAZ	Nil
6.	AMP	Nil
7.	AMS	6 mm
8.	C	8mm

Cultural characteristics observed after incubation at 35-37°C of 18-48 hours incubation. The organism is *Staphylococcus aureus*(Figure 3).

Figure 3: Analysis the MIC (Minimum Inhibitory Concentration) for various type of Antibiotics shown in Table 2.

Pure Culture (Streak) plate method:

Culture characteristics observed after incubation at 30-35°c for 18-72 hours. The organism is*Escherichia coli*(Figure 4).

Figure4: Streak plate *E. coli* bacteria

Cultural characteristics observed after incubation at 35-37°C of 18-48 hours incubation. The organism is *Staphylococcus aureus*(Figure 5).

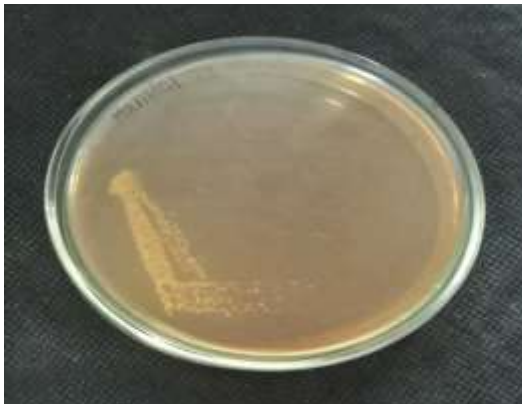

Figure 5: *Staphylococcus aureus*

Cultural characteristics observed after incubation 35°C for 18-48 hours. The organism is *Escherichia coli*(Figure 6, 7,8).First tube is control and second tube is sample.

Figure 6: *E. coli*

Identification of Species:

Table 3: Identification of Bacteria based on Morphology

S.No	Name of the medium	Growth	Colour	Morphology	Organism
1.	Macconkey agar	Positive	Pink- red with bile precipitate	Mucoid	*Escherichia coli*
2.	Mannitol salt agar	Positive	Yellow, white colonies surrounded by yellow zone	Mucoid	*Staphylococcus aureus*
3.	Triple salt iron	Positive	Yellowing of the medium	Mucoid	*Escherichia coli*

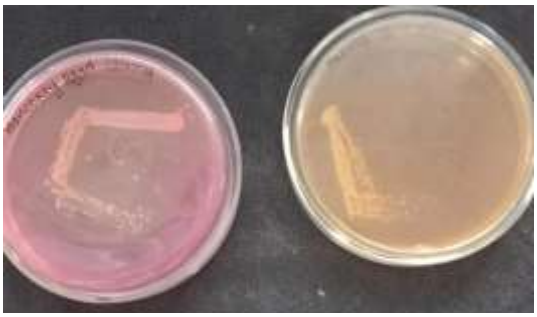

Figure 7: ***E. coli andS. aureus***

Figure 8: *Staphylococcus aureus*

Calculation

Mic = Control OD – Sample OD ×100/ Control OD

Mic = 0.256 +0.250+0.258/3

= 0.254

10 µl = 0.256 – 0.192 ×100/0.254

= 25.196

20 µl = 0.250 –0.173 ×100/0.254

= 30.314

30 µl = 0.258 – 0.124 ×100/0.254

= 52.755

Table 4: Post Incubation Result of ELISA

S.No	Description	Test I	Test II	Test III
1.	Broth	0.001	0.055	
2.	Broth+ culture	0.256	0.250	0.258
3.	Broth + culture +drug (added different concentration)	10µl 0.192	20µl 0.173	30µl 0.124

Plant Extract:

Figure 9: A - Plant extract of *Andrographis paniculate,* B- Control, C - egg with plant extract

Table 5: Concentration of organism grown in egg dipped (Plant Extract) LB Broth

S.No	Days	Blank	Control	Sample
1.	0 Day	0.00	0.00	0.00
2.	24 hrs	0.00	0.186	0.010
3.	48 hrs	0.00	0.308	0.193
4.	72 hrs	-	-	-

Agarose Gel Electrophoresis

DNA fragments were resolved in the agarose gel. The DNA mobility was based on their molecular weight. Higher molecular fragments were found near to the well, while lower molecular fragment migrated a greater difference away from well. Hence the bacterial DNA was observedand documented.

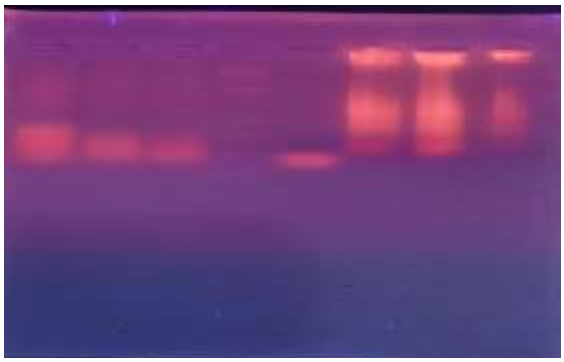

Figure 10: Amplified DNA from Multi-resistant Bacteria

ACKNOWLEDGEMENTS

Authors would like to express thanks to Management, AJK College of Arts and Science, and Centre for Bioscience and Nanoscience Research, Coimbatore for providing facilities to carry out this research work.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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