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Prevalence, Biochemical Characteristics and Antibiotic Susceptibility of Pathogenic Bacteria in Poultry Litter

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Abstract: The quality, quantity and management of litter affects the growth and performance of broiler. It also has been reported to acts as a reservoir of microbes that may infect, reinfect, mutate and may cause disease therefore this study was designed for analysis of prevalence, biochemical characteristics and antibiotic susceptibly of microbes isolated from different poultry litters used in Sindh province of Pakistan. A total of (n) =66 litter samples including rice husk (33), wooden bran (21) and sand (12) were collected from different broiler farms. The prevalence of bacteria were recorded as 36(54.54%) samples were positive with rice husk 20(60.60%), wooden bran 10(47.61%) and sand 6 (50.00%).The isolated bacteria were identified as *E. coli* in 9(45.0%) samples, followed by *L.monocytogen*in 3(15.0%), *S. arizonae*in 3(15.0%), *S. aureus*in 3(15.0) and *P.aeroginosa*in 2(10.0%) samples collected from rice husk litter. Similarly, *E. coli*in 4(40.0%) samples, S.*arizonae*in 3(30.0%), *S. aureus*in 3(30.0%) and *P.aeroginosa*1(10.0%) were isolated from wooden bran, respectively. However, from sand litter only *E. coli*in5(83.33%) samples and *S.arizonae*in 1(16.67%) samples were isolated.

The morphological characteristics show that *E. coli*were observed as cocco-bacilli, short rods & straight rods, arranged either singly or in pairs, non-motile and gram negative. Similarly, *L. monocytogenes*were coccoid rods, arranged singly & in short chains, motile and gram positive. *P. aeruginosa* were found in short to long cylinder or singly curved rods, arranged in pairs and short chains, motile and gram negative. Similarly, *S. arizonae*were observed as rod to cocco bacilli shape, arranged single, pairs, tetrads, short curved chains & in few clusters, G -ve. However, the shape of *S. aureus* was round and grape like structure, arranged as in pairs, tetrads, short & irregular clusters, non-motile and gram positive.

Biochemical analysis revealed that *E. coli*, *L. monocytogen*, *S. arizonae* and *S. aureus* were urease negative, catalase positive, produced acidic slant and acidic butt whereas *P. aeroginosa* was urease and catalase positive and produced alkaline slant and butt. Sugar fermentation indicated that *E. coli*, *L. monocytogen*, *P. aeroginosa*, *S. arizonae* and *S. aureus* was ferment glucose, mannitol and mannose but failed to ferment dulcitol except *S.*aureus.

Antibiotic sensitivity test shows that *E. coli* and *L. monocytogen*were highly sensitive to chloramphenicol and gentamycin, *P. aeruginosa* and *S.aureus* was highly sensitive to sulphamethazole and tetracycline while *S. arizonae* were sensitive to ampicillin and neomycin. antibiotic sensitivity further revealed multiple drug resistant or moderate sensitivity of *E. coli* with amoxicillin and sulphamethaoxazole, *S. arizonae* were particularly resistant to amoxicillin, chloramphenicol, sulphamethaoxazole and tetracycline. Similarly, *S. aurus* were moderately sensitive to ampicillin, amoxicillin, gentamycin and chloramphenicol.

Keywords: Litter, Pathogenic Bacteria, Biochemical Behavior and Antibiotic Sensitivity

1. INTRODUCTION

Poultry industry plays a key role in the economy of Pakistan. Poultry production has been increased due to intensive farming, genetic improvement and environmental control modern farming (Sadiq, 2004). Despite rapid growth it is influenced by various diseases such as respiratory disease, broiler dermatitis (Berg and Lund, 2003), *E. coli* and Salmonella species (Paliwal *et al.*, 2008). Incidences of these disease reduce production as well as cause serious food hygiene (Hughes and Black, 1974) making the broiler welfare and management necessary. It has been found that the growth rate, rearing, intensity of light, density of birds on a farmfeeding systems, controlled environment, and litter are an important factor that affect broiler performance (Robins and Phillips, 2011).

Broiler birds are commonly reared on floors that are covered with litter. Litter plays a vital rolein providing required thermal insulation, act as absorbent that absorb spilled liquid and excreted material, drying pens by reducing contact between birds and floor (Shepherd and Fairchild, 2010). Being a protective medium litter should be absorptive, light or medium weight, economical, non-hazardous and reusable.

Cost and availability of litter varies from region to region.Generally,litter materialsused aresaw dust and rice hulls although peanut hulls, sand and soybean straw were occasionally used (de Avila *et al.*, 2008).Research has found that sawdust has high capability of water absorption capacity and proved as familiar litter material (Dan Shao *et al.*, 2015). Moreover, sawdust, rice husk, sugarcane bagasse and wheat straw has been used as a litter material for broilers (Monira *et al.*, 2003).

Although, pathogenic bacteria affect broilerlive weight gain and performance. These bacteria either

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directly or indirectly affect the performance of broiler. Studies has been conducted on various litter materials, thickness in summer (Erensayin, 1991). It has been found that thickness of litter mainly influences humidity, environmental conditions and weight gain (DanShao *et al*., 2015). Similarly, continually standing on humid and watery litter badly harm the footpad and lead the footpad disease associated ulceration, provide a better medium for bacterial growth and act as a predisposing factor to develop pyogenic bacteria andinfections caused by *E. coli* (Shepherd and Fairchild, 2010). Research has found that total bacterial count in poultry litter samples consisting of rice husk and identified several pathogenic bacteria including *E. coli*, *salmonella arizonae, listeria monocytogens* and *S.ylococcusaureus* (Schochen-iturrino *et al*., 1996). Similarly, poultry litter were contained *salmonella spp* and *E. coli* (Rezende *et al*., 2001). It has been found that poultry litter comprise a variety of pathogenic bacteria including clostridia spp (37%), *E. coli* (49%), *listeria* (20%) *S.ylococcus* (25%) and *salmonella spp* (12.3%) respectively. *Escherichia coli* is highly prevalent and increasing every year that cause serious illness and death in birds as well as in humans associated with >80% of urinary tract infections (Foxman, 2002)

Keeping in view scare knowledge available on prevalence of pathogenic bacteria in poultry litter, biochemical behavior, antibiotic sensitivity and morphological characteristicsthis study is conducted to know prevalence of pathogenic bacteria, microbial susceptibility, colony characteristics and biochemical behavior of various bacteria isolated from poultry litter.

2. MATERIALS AND METHODS

Samples collection, transportation and handling:

A total of (n=66) litter samples including rice husk (33), wooden bran (21) and sand (12) were collected from different broiler farm surrounded by Karachi. Litter samples were collected in sterile crew caped bottles from randomly selected site of poultry house (the floor, litter piles at the entrance of poultry farm and water lines). The samples were brought to laboratory of Sindh Institute of animal health for further analysis.

Isolation and identification of selected bacteria:

Cultural methods for detection of bacterial species involves a non-selective pre-enrichment, followed by selective enrichment and plating onto selective and differential agars.A total of 1gram of solid material was added to the 9ml of nutrient broth, incubated aerobically at 37◦C for 24hrs in microbiological incubator (Qualitron), after 24hrs of incubation, the broth culture was than streaked on selective and differential agar, streaked on EMB agar (Oxoid), Mac Conkey's agar (Oxoid), bismithsulphate agar (Oxoid), mannitolsalt agar (Oxoid), blood agar (Oxoid), cetramideagar (Oxoid) and listeria identification agar base (PALCAM, Himedia). Suspected colonies were confirmed through Gram's staining and biochemical analysis.

Gram's staining:

The isolated bacteria i-e; *salmonella*, *E. coli*, *Pseudomonas*, *S. aureus* and *listeria* were characterized morphologically using Gram's staining method as described by (Merchant and Packer., 1967).

Biochemical analysis test:

Isolated organisms showing characteristics colony morphology of *Salmonella*, *E. coli*, *Pseudomonas*, *S. aureus* and *listeria* were subjected to biochemical test such as sugar fermentation, indole, catalase, MR-VP test, motility test, citrate utilization test using Simmons citrate agar and carbohydrate fermentation test as described by (Barrow and Feltham 2003)

Motility test:

The motility test was performed to differentiate motile bacteria from non-motile bacteria as described by (Cheesbrough., 1984).

Antibiotic susceptibility of bacteria:

All isolates were tested for antibiotic susceptibility using Kirby Bauer disc diffusion assay (Bauer *et al*., 1966). The results of antibiotic sensitivity test were shown in table-VII.

3. RESULTS

A total of 66 litter samples including rice husk (33), wooden bran(21) and sand (12) were collected and examined for bacterial isolation. The results revealed that total samples 36 (54.54%) were found positive including rice husk 20(60.60%), wooden bran 10(47.61%) and sand 6 (50.00%) respectively **(Table I).**

Table I.- Prevalence percentage pathogenic bacterial species in several types of litter.

Type of poultry litter	Number of sample	No of positive samples	% of positive samples
Rice husk	33	20	60.60
Wooden	21	10	47.61
bran	12	10	50.00
Sand	**66**	**36**	**54.54**
Total			

Identification of bacterial species revealed that *E. coli* 9(45.0%), followed by *L.monocytogen* 3(15.0%), *S. arizonae* 3(15.0%), *S. aureus* 3(15.0) and *P.aeroginosa* 2(10.0%) in rice husk. Four species were found on wooden bran i-e; *E. coli* 4(40.0%), S.*arizonae* 3(30.0%), *S. aureus* 3(30.0%) and *P.aeroginosa* 1(10.0%). Two species were found on sand *E.coli* 5(83.33%) and*S. arizonae*1(16.67%) **(Table 2).**

Table 2.- Litter samples found positive for various bacterial species.

Bacterial Species	Rice husk		Wooden bran		Sand	
	No. of +ve samples	% of +ve samples	No. of +ve samples	% of +ve samples	No. of +ve samples	% of +ve samples
E. coli	9/20	45.0	4/10	40.0	5/6	83.33
L.monocytogen	3/20	15.0	0/10	00.0	0/0	0.00
P. aeroginosa	2/20	10.0%	1/10	10.0	0/0	0.00
S. arizonae	3/20	15.0%	3/10	30.0	1/6	16.67
S. aureus	3/20	15.0%	2/10	20.0	0/0	0.00

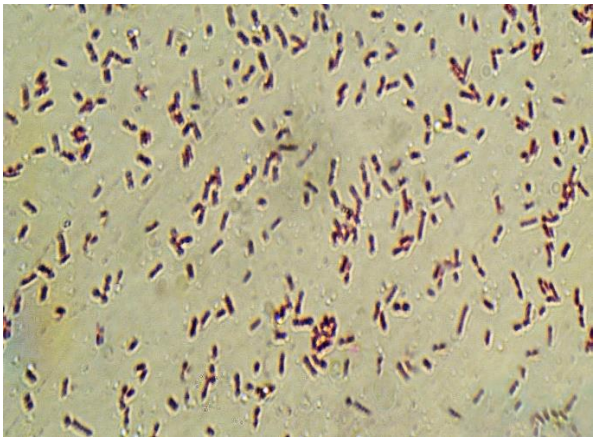
Results revealed the morphological and cultural characteristics of various bacteria such as *E. coli* were observed ascocco-bacilli, short rods & straight rods, arranged either singly or in pairs, non-motile and gram negative. On blood agar non-hemolytic grey white, moist listening, opaque, circular, convex with entire edge, on MacConkey's agar, it produces fermentative pink colonies. *Listeria monocytogenes* coccoid rods, arranged singly & in short chains, motile and gram positive. On B / A colonies were beta hemolytic with grayish white, convex entire margin and circular in shape, on M/A colonies with color, medium, small, circular, entire smooth, sticky and translucent. *P. aeruginosa* were found in short to long cylinder or singly curved rods, arranged in pairs and short chains, motile and gram negative. On B / A colonies were beta-hemolytic, on M/A it did not produce fermentative colonies. *S.arizonae* revealed rod to cocco bacilli shape, arranged single, pairs, tetrads, short curved chains & in few clusters. On B / A colonies were non-hemolytic, on M/A, it produces fermentative colonies. *S. aureus* wasround, grape like structure, pairs, tetrads, short & irregular clusters, non-motile and gram positive. Culturally it produces entirely convex and beta-hemolytic on B/A whereas on M/A no growth was observed **(Table 3,4 and Fig. I).**

Table 3. Morphological and staining characteristics of bacterial species prevalent of poultry litter.

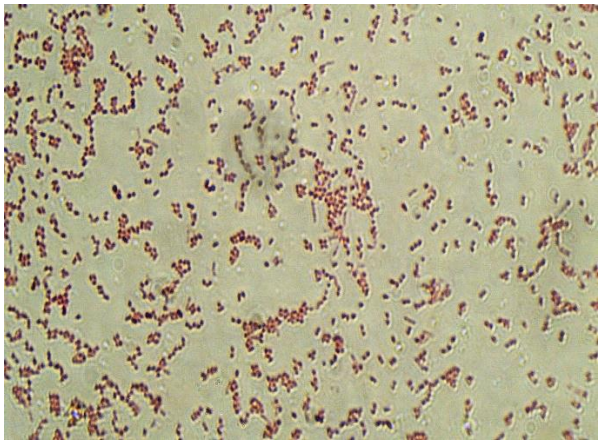
Bacterial species	Shape	Arrangement	Motility	Staining
E. coli	Cocco-bacilli, short rods & straight rods	Single & in pairs	Non-motile	G-ve
L. monocytogen	Coccoid rods	Single & short chains	Motile	G+ve
P. aeruginosa	Short to long cylinder or singly curved rods	Occur in pairs and short chains	Motile	G-ve
S. arizonae	Small rod or cocco bacilli	Single, pairs, tetrads, short curved chains & in few clusters	Motile	G-ve
S. Aureus	Cocci	Grape like structure, pairs, tetrads, short & irregular clusters	Non-motile	G+ve

Table 4. colony characteristics of various bacterial species isolated from poultry litter.

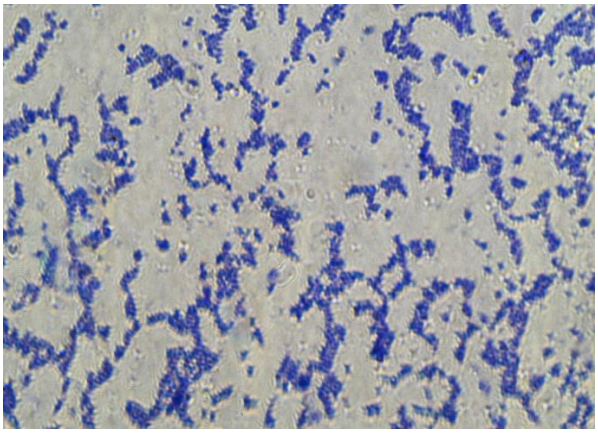
Bacterial species	Colony characteristics	Colour
E. coli	On B / A colonies were non-hemolytic grey white, moist listening, opaque, circular, convex with entire edge, on M/A fermentative pink colonies	Grey and shiny
L. monocytogenes	On B / A colonies were beta hemolytic with grayish white, convex entire margin and circular in shape, on M/A colonies with colour, medium, small, circular, entire smooth, sticky and translucent	Grayish white
P. aeruginosa	On B / A colonies were beta-hemolytic, on M/A it did not produce fermentative colonies	Motile
S. arizonae	On B / A colonies were non-hemolytic, on M/A, it produces fermentative colonies	Motile
S.aureus	On B / A colonies were entire convex and beta-hemolytic whereas on M/A no growth was observed	Non-motile



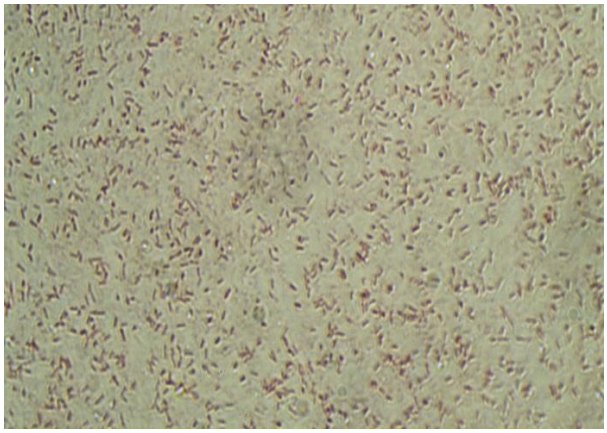
E. coli: G-ve, coccobacilli, short rods on 100X



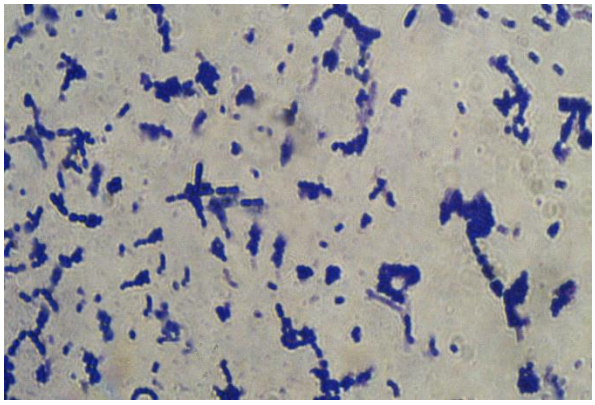
S. arizonae: G-ve, small rods,Single, pairs, tetrads & short curved chains on 100X



S. aurus: G+ve, cocci, Grape like structure, pairs, tetrads, short & irregular clusters on 100X



Pseudomonas: cylinder or singly curved rods, occur in pairs and short chains



Listeria spp: G+ve, cocci, arranged in chains, 100X

Fig. I:- Morphology and colony characteristics of different bacteria.

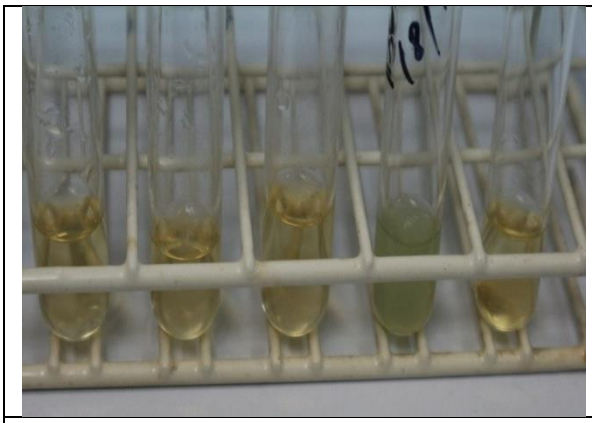


Fig. I a. growth of various bacteria in Nutrient Broth (Oxide) indicated by turbidity.

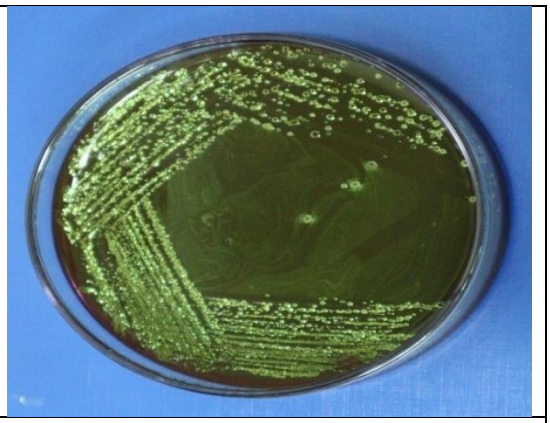


Fig I b. Green metallic sheen of *E. coli* on EMB agar (Oxide).

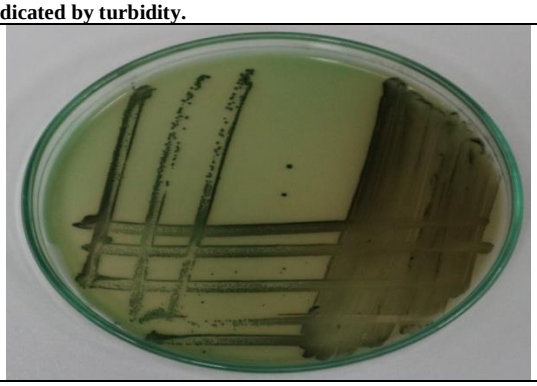


Fig. Ic. black colonies of *Salmonella spp* shows H_2S production on bismithsulphite agar (Oxide)

Fig. Id *S. aurus* colonies shows yellow halo on MSA agar (Oxide).

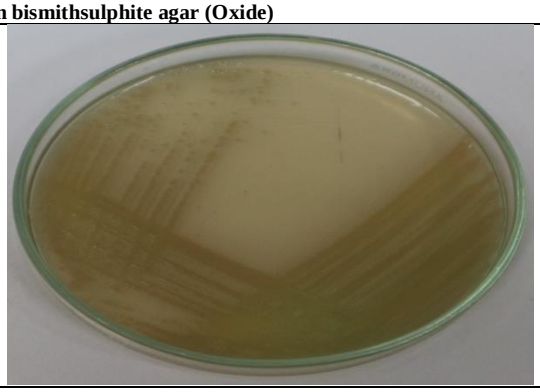


Fig. I. *Pseudomonas* pigmentation on citermide agar (Oxide).

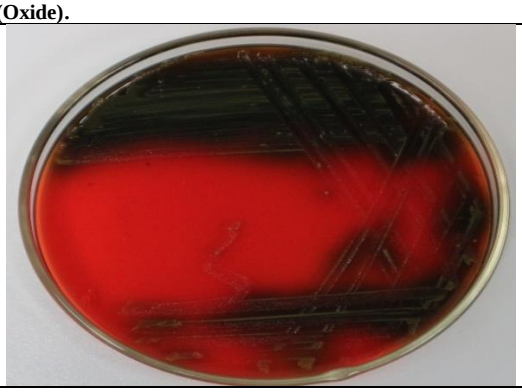


Fig. If. Grey green colonies of listeria spp. with black center and black halo on PALCAM agar (Himedia).

Fig. I. Growth of various bacteria on selective media:

Results of biochemical analysis and sugar fermentation has revealed that *E. coli* was found catalase positive and oxidase negative, exhibited A/A (acidic slant and acidic butt). It did not interact with urease, voges-proskauer and citerate. Correspondingly it fermentsmaltose, mannitol, xylose,glucose, mannose, and inositol but did not ferment inositol, creatinine and dulcitol. *Listeria monocytogenes* was found positive for catalase and TSI and produced A/A. It interacts with methyl red and voges-proskauer, negative for oxidase, urase, indole and Simon's citrate. Ferment glucose, mannose, mannitol, xylose and inositol. *P. aeruginosa* found positive for oxidase,catalase, urease and citrate. It produced K/K (alkaline slant and alkaline butt) and ferment glucose, mannose and mannitol. *S. arizonae* produced A/A and H2S in TSI, positive for methyl red and catalase. It fermentsxylose, inositol, mannitol, mannose, glucose, and maltose while do not ferment creatinine and dulcitol. *S. aureus* was positive for catalase and negative for oxidase.It produced A/A, ferment maltose, inositol, glucose, mannose, xylose, dulcitol and mannitol respectively **(Table 5 and 6 and Fig 2,3, and 4)**

Table 5. Bio-chemical properties various bacterial species isolated from poultry litter.

Bacterial species	Sc	MR	VP	Ind	H2S	Cat	Oxid	Coag	TSI	Hemo	Urease
E. coli	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-	A/A	-	-ve
L. monocytogen	-ve	+ve	+ve	-ve	-ve	+ve	-ve	-	A/A	B	-ve
P. aeroginosa	-ve	-ve	-ve	-ve	-ve	+ve	+ve	-	K/K	B	+ve
S. arizonae	+ve	+ve	-ve	-ve	+ve	+ve	-ve	-	A/A	-	-ve
S. aureus	-ve	-	-	-	-ve	+ve	-ve	+ve	A/A	B	-ve

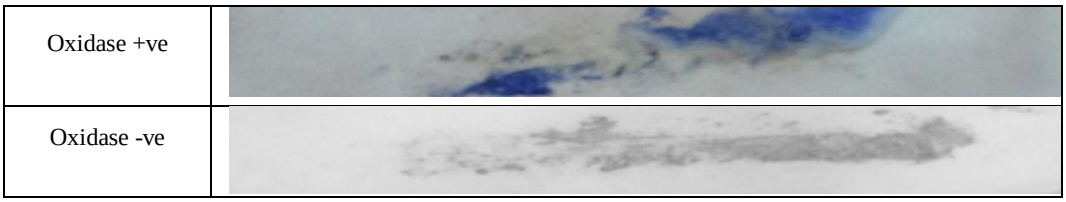


Fig.. 2:- Oxidase test for various bacteria.

Fig. 3 Catalase test for various bacteria.

Control | *S. arizonae* with H2S production | *S. arizonae* without H2S production | *Pseudomonas* | *E. coli* | *S. aurus* | *L.monocytogene*

E. coli, *L. monocytogen*, *P. aeroginosa*, *S. arizonae* and *S. aureus* was found to ferment glucose, maltose, mannitol and mannose and do not ferment dulcitol except *S. aureus*.

Fig. 4 Triple sugar iron test.

Table 6. Sugar fermentation properties of various bacteria isolated from poultry litter

Bacterial species	Glucose	Maltose	Mannitol	Inos	Mannose	Xylose	Creatinin	Dulcitol
E. coli	+ve	+ve	+ve	-ve	+ve	+ve	-ve	-ve
L. monocytogen	+ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve
P. aeruginosa	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
S. arizonae	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
S. aureus	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve

Results of antibiotic sensitivity test revealed that *E. coli* was highly sensitive to chloramphenicol followed by gentamycin and neomycin, moderately susceptible to ampicillin and resistant to amoxicillin, kanamycin, tetracycline and sulphamethoxazole. *L. monocytogenes* found highly sensitive to gentamycin followed by chloramphenicol, kanamycin, tetracycline, ampicillin and neomycin while resistant to amoxicillin and sulphamethoxazole. Moreover, *P. aeroginosa* was highly sensitive to sulphamethoxazole, followed by tetracycline and gentamycin. *Salmonella arizonae* was found moderately sensitive to gentamycin, ampicillin, kanamycin and neomycin while resistant to amoxicillin, chloramphenicol, sulphamethoxazole and tetracycline. *S. Aureus* was found highly sensitive to tetracycline and sulphamethoxazole while moderately sensitive to ampicillin, amoxicillin, gentamycin and chloramphenicol **(Table 7).**

Table 7. Antibiotic drug sensitivity of pathogenic bacteria found on poultry litter.

Antibiotic disc used	*E. coli*		*L.monocytogen*		*P.aeroginosa*		*S.arizonae*		*S.Aureus*	
	Zone of inhibition	Indication of sensitivity	Zone of inhibition	Indication of sensitivity	Zone of inhibition	Indication of sensitivity	Zone of inhibition	Indication of sensitivity	Zone of inhibition	Indication of sensitivity
Amoxicillin	2mm	+	0mm	-	9mm	+++	0mm	-	10mm	+++
Ampicillin	6mm	++	10mm	+++	11mm	++++	10mm	+++	8mm	+++
Chloramphenicol	18mm	++++	11mm	++++	8mm	+++	0mm	-	6mm	++
Gentamycin	11mm	++++	18mm	++++	12mm	++++	11mm	++++	9mm	+++
Kanamycin	2mm	+	11mm	+++	10mm	+++	10mm	+++	10mm	+++
Neomycin	10mm	+++	7mm	++	11mm	++++	10mm	+++	10mm	+++
Sulphamethazole	0mm	-	10mm	+++	18mm	++++	0mm	-	14mm	++++
Tetracycline	0mm	-	11mm	++++	12mm	++++	1mm	+	14mm	++++

Absence of clear zone around disc -, Clear zone with 1-3mm diameter +, Clear zone with 3-7mm diameter ++, Clear zone with 7-11mm diameter+++, Clear zone with 11-18mm diameter++++.

4. DISCUSSION

It has been found that bedding material a key impact on growth and development of broiler industry. Despite of this it acct as a source of infection. Current study has examined several types of poultry litter and found 36 (54.54%) positive for bacterial contamination. Of these 66 samples, 33, 21, 12 were rice husk, wooden bran and sand.Results revealed that *E. coli* 9(45%) followed by 3(15), 3(15%), 2(10), 3(15) for *Listeria monocytogenes*, *Pseudomonas aroginosa*, *Salmonella arizonae* and *S.ylococcusaureus*. Followed by wooden bran and sand. This might be attributed to accumulation of moisture content of bedding material that plays a vital role bacterial multiplication. Results of current study agree with Rezende *et al.* (2001) who have reported presence of higher count of *salmonella* and *E. coli* in poultry litter. Fries *et al.* (2005) who have studied composition of straw and wooden shaving of poultry litter by rearing day-old chicks. They found most of gram positive bacteria were arranged in irregular rods.

Percentage of bacteria on litter as, *E. coli* 9(50%) in rice husk followed by 5(27.78%) and 4(22.22%) in sand and wooden branrespectively. *Listeria monocytogenes* 3 (15.0%), *Salmonella arizonae* 3 (15.0%), *S.ylococcusaureus* 3 (15.0%) and *P.aeroginosa* 2(10.0%) respectively in rice husk. Moreover, *E. coli* 4(40.0%), S.*arizonae* 3(30.0%), *S.aureus* 3(30.0%) and *P.aeroginosa* 1(10.0%) in wooden bran while two species were found on sand *E. coli* 5(83.33%) and *S. arizonae* 1(16.67%) respectively. Correspondingly Ogonwaski*et al.* (1995) who have repoted *clostridia spp, E. coli, listeria, S.ylococcus and salmonella spp* 37%, 49%, 20%, 25%, and 12.3% respectively. Similarly, phenotypic charateristics and biochemical testes of *E. coli*, *Listeria monocytogenes*, *Pseudomonas aroginosa*, *Salmonella arizonae* and *S.ylococcusaureus*are in correlation with Rind (2001), Mahanjan *et al.* (2003) and Fazalani (2005) who have found similar characteristics of these bacterial isolates.

Results of antibiotic sensitivity of bacterial isolates of litter revealed that E. coli, listeria monocytogenes were highly susceptible to chloramphenicol, gentamycin and neomycin while resistant to amoxicillin and tetracycline. *Pseudomonas aeruginosa* and *S.ylococcusaureus* were found highly sensitive to sulphamethazole and tetracycline while moderately sensitive to other drugs. *Salmonella arizonae* was found moderately susceptible to ampicillin, kanamycin and neomycin while resistant to rest of drugs. Similarly, Wilson, (2004) who has reported salmonella spp. isolated from poultry birds were found resistant to ampicillin, tetracycline, and gentamycin. Sensitivity of *Pseudomonas aeruginosa* are highly corelated with Rind and shaikh (2001) who have found it is sensitive to gentamycin, chloramphenicol,tetracycline and sulphamethazole. Correspondingly, results of *S.ylococcusaureus* are in agreement with Rind and Khan (2000) hasreported that it is susceptible to tetracycline, sulphamethazole and gentamycin.

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