**CHAPTER: 4**

**RESULTS AND DISCUSSIONS**

**RESULTS:**

**Table-4.1:** **Culture of samples on MacConkey and EMB Agar for *E. coli* isolation and identification**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample No.** | **MacConkey** | **EMB** | **Microscopic features** |
| H1 | +VE | +VE | Gram-negative, rod |
| H2 | +VE | +VE | Gram-negative, rod |
| H3 | +VE | +VE | Gram-negative, rod |
| H4 | +VE | +VE | Gram-negative, rod |
| H5 | +VE | +VE | Gram-negative, rod |
| H6 | +VE | +VE | Gram-negative, rod |
| V1 | +VE | +VE | Gram-negative, rod |
| V2 | +VE | +VE | Gram-negative, rod |
| V3 | +VE | +VE | Gram-negative, rod |
| V4 | +VE | +VE | Gram-negative, rod |
| V5 | +VE | +VE | Gram-negative, rod |
| S1 | +VE | +VE | Gram-negative, rod |
| S2 | +VE | +VE | Gram-negative, rod |
| S3 | +VE | +VE | Gram-negative, rod |
| S4 | +VE | +VE | Gram-negative, rod |
| S5 | +VE | +VE | Gram-negative, rod |

+VE = Positive -VE = Negative

Culture of effluent on MacConkey agar for the isolation of *E. coli* were able to produce bright pink colonies (non-mucoid) due to fermentation of lactose, while lactose negative organisms (*Salmonella, Shigella*) have only peptone as energy source were colorless. Similarly, sub-cultured on EMB agar the colonies had very dark and almost black colonies when observed directly against the light. By reflected light, a green sheen were seen which is due to the precipitation of methylene blue in the medium and the very high amount of acid produced from lactose fermentation are the characteristics to *E. coli.*

**Microscopic study by Gram’s staining method**

In Gram’s staining, microscopically positive colonies able to revealed Gram-negative, rod shaped bacteria.

**Table-4.2: Culture of samples on XLD and BGA agar for *Salmonella* isolation and identification**

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample No.** | **XLD** | **BGA** | **Microscopic features** |
| H1 | -VE | -VE | ND |
| H2 | -VE | +VE | Gram-negative, pink colored, small rod |
| H3 | -VE | -VE | ND |
| H4 | -VE | -VE | ND |
| H5 | +VE | +VE | Gram-negative, pink colored, small rod |
| H6 | -VE | +VE | Gram-negative, pink colored, small rod |
| V1 | -VE | -VE | ND |
| V2 | +VE | +VE | Gram-negative, pink colored, small rod |
| V3 | -VE | -VE | ND |
| V4 | -VE | -VE | ND |
| V5 | -VE | -VE | ND |
| S1 | -VE | -VE | ND |
| S2 | -VE | -VE | ND |
| S3 | -VE | -VE | ND |
| S4 | -VE | -VE | ND |
| S5 | -VE | -VE | ND |

+VE = Positive -VE = Negative ND: Not detected

Colonies were isolated as positive on the basis of characteristic colony color and morphology cultured on XLD and BGA agar from which positive isolates were found in sample no H5, H6 and V2. On BGA, *Salmonella* colonies were surrounded by a pink zone, whereas on XLD agar, the colonies appeared as black centered because of H2S production. Non-*Salmonella* colonies appeared white with yellow background on XLD plates, and on BGA plate’scolonies were white.

**Microscopic study by Gram’s staining method**

Gram-negative, pink colored small rod shaped bacteria were found from sample number H2, H5, H6 and V2. Based on the characteristic growth and colony color, it assumed that organisms are *Salmonella spp.*

**Table-4.3: Culture of samples on Mannitol Salt Agar for *Staphylococcus* isolation and identification**

|  |  |  |
| --- | --- | --- |
| **Sample**  | **Mannitol Salt Agar** | **Microscopic features** |
| H1 | +VE | Gram-positive, grape like cluster |
| H2 | -VE | ND |
| H3 | +VE | Gram-positive, grape like cluster |
| H4 | +VE | Gram-positive, grape like cluster |
| H5 | +VE | Gram-positive, grape like cluster |
| H6 | +VE | Gram-positive, grape like cluster |
| V1 | +VE | Gram-positive, grape like cluster |
| V2 | +VE | Gram-positive, grape like cluster |
| V3 | -VE | ND |
| V4 | -VE | ND |
| V5 | -VE | ND |
| S1 | +VE | Gram-positive, grape like cluster |
| S2 | +VE | Gram-positive, grape like cluster |
| S3 | +VE | Gram-positive, grape like cluster |
| S4 | -VE | ND |
| S5 | +VE | Gram-positive, grape like cluster |

+VE = Positive -VE = Negative ND: Not detected

The Staphylococcus aureus ferments mannitol and turned the medium yellow. The colonies were characterized as round, smooth and glistening that was positive in sample H1, H3, H4, H5, H6, V1, V2, S1, S2, S3 and S5 respectively, as mannitol salt agar is selective media for *Staphylococcus spp.*

**Microscopic study by Gram’s staining method**

The positives isolated from mannitol salt agar microscopically detected as Gram-positive, coccid as clustered of grapes.

**Table-4.4: Indole test for *E. coli* isolated from samples**

|  |  |
| --- | --- |
| **Sample No.** | **Indole test** |
| H1 | +VE |
| H2 | +VE |
| H3 | +VE |
| H4 | +VE |
| H5 | +VE |
| H6 | +VE |
| V1 | +VE |
| V2 | +VE |
| V3 | +VE |
| V4 | +VE |
| V5 | +VE |
| S1 | +VE |
| S2 | +VE |
| S3 | +VE |
| S4 | +VE |
| S5 | +VE |

 +VE = Positive

Tests results positive for the presence of Indole as indicated by the red reagent layer after the addition of Kovács reagent were found in all samples from which the positive *E. coli* were isolated.

**Table-4.5: TSI slant for isolated *E. coli* and *Salmonella spp.***

|  |  |
| --- | --- |
| Sample No. | TSI slant |
| *E. coli* **(slant/butt)** | *Salmonella* **(slant/butt)** |
| H1 | A/A,G | ND |
| H2 | A/A,G | K/A |
| H3 | A/A | ND |
| H4 | A/A | ND |
| H5 | A/A,G | K/A,G,H2S |
| H6 | A/A,G | K/A,G,H2S |
| V1 | A/A,G | ND |
| V2 | A/A,G | K/A,G,H2S |
| V3 | A/A,G | ND |
| V4 | A/A | ND |
| V5 | A/A,G | ND |
| S1 | A/A,G | ND |
| S2 | A/A,G | ND |
| S3 | A/A,G | ND |
| S4 | A/A,G | ND |
| S5 | A/A,G | ND |

A/A = Yellow/Yellow; A/A, G = Yellow/Yellow with bubbles (gas); K/A = Red/Yellow; K/A, G, H2S = Red/Yellow with bubbles and black precipitate; ND = Not detected

In case of suspected *E. coli* the sample number H1, H2, H5, H6, V1, V2, V3, V5, S1, S2, S3, S4 and S5 were shown yellow slant and yellow butt with gas production, the Sample number H3, H4 and V4 were shown yellow slant and yellow butt without any bubble formation. In case of suspected *Salmonella,* the sample number H2 shown red slant and yellow butt. And the samples number H5, H6 and V2 were shown red slant yellow butt with bubbles (gas) and black precipitation that was confirmatory to *Salmonella.*

**Table-4.6.a: CS-test for isolates of *E. coli***

|  |  |
| --- | --- |
| **Sample**  | **Antibiotic disc used** |
| **AMP** | **CIP** | **CL** | **DO** | **E** | **ENR** | **GEN** | **N** | **PF** | **TA** |
| **H1** | R | R | R | R | R | R | S | S | R | R |
| **H2** | R | R | R | R | R | R | R | S | R | R |
| **H3** | R | R | R | R | R | R | R | R | R | R |
| **H4** | R | R | R | I | R | R | S | I | R | R |
| **H5** | R | R | R | R | R | R | S | I | R | R |
| **H6** | R | R | R | R | R | R | R | R | R | R |
| **V1** | R | R | R | R | R | R | R | R | R | R |
| **V2** | R | S | S | R | R | R | I | R | R | R |
| **V3** | R | R | R | R | R | R | I | R | R | R |
| **V4** | R | S | S | I | R | R | S | R | R | R |
| **V5** | R | S | S | R | R | R | S | S | R | I |
| **S1** | R | R | R | R | R | R | R | R | R | R |
| **S2** | R | R | R | R | R | R | R | R | R | R |
| **S3** | R | R | R | R | R | R | R | I | R | R |
| **S4** | R | R | R | R | R | R | R | I | R | R |
| **S5** | R | R | R | R | R | R | R | I | R | R |

AMP=Ampicillin, CIP=Ciprofloxacin, CL = Colistin, DO = Doxycycline, E = Erythromycin, ENR=Enrofloxacin, GEN=Gentamycin, N=Neomycin, PF=Pefloxacin, TA= Oxytetracycline

R=Resistance I=Intermediate S=Sensitive

**Table-6.b: Prevalence of hospitals and slaughterhouses isolates *E.coli***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibiotic** | **Pattern** | **Medical Hospital** | **Veterinary Hospital** | **Slaughterhouse** |
| **AMP** | Resistance | 6 (100%) | 5 (100%) | 5 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |
| **CIP** | Resistance | 6 (100%) | 2 (40%) | 5 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 3 (60%) | 0 (0%) |
| **CL** | Resistance | 6 (100%) | 2 (40%) | 5 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 3 (60%) | 0 (0%) |
| **DO** | Resistance | 5 (83%) | 4 (80%) | 5 (100%) |
| Intermediate | 1 (17%) | 1 (20%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |
| **E** | Resistance | 6 (100%) | 5 (100%) | 5 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |
| **ENR** | Resistance | 6 (100%) | 5 (100%) | 5 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |
| **GEN** | Resistance | 3 (50%) | 1 (20%) | 5 (100%) |
| Intermediate | 0 (0%) | 2 (40%) | 0 (0%) |
| Sensitive | 3 (50%) | 2 (40%) | 0 (0%) |
| **N** | Resistance | 2 (33%) | 4 (80%) | 2 (40%) |
| Intermediate | 2 (33%) | 0 (0%) | 3 (60%) |
| Sensitive | 2 (33%) | 1 (20%) | 0 (0%) |
| **PF** | Resistance | 6 (100%) | 5 (100%) | 5 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |
| **TA** | Resistance | 6 (100%) | 4 (80%) | 5 (100%) |
| Intermediate | 0 (0%) | 1 (20%) | 0 (0%) |
| Sensitive | 0 (0%) | 0(0%) | 0 (0%) |

The medical hospital *E. coli* isolated from H1 sample was resistant to AMP, CIP, CL, DO, E, ENR, PF and TA respectively, but were sensitive to GEN and N. Isolates from H2 was sensitive to N and were resistant to nine other tested antibiotics. *E. coli* from H3 was resistant to all ten tested antibiotics. E. coli isolated from sample number H4 was resistant to AMP, CIP, CL, E, ENR, PF and TA respectively, sensitive was only to GEN and intermediate sensitive to DO and N. Sample number H5 isolates was sensitive to only GEN and intermediate sensitive to N but were resistant to tested eight other antibiotics. Similarity to H3 was observed in sample number H6. The levels of resistance exhibited by isolates to specific antibiotics was 100% resistance to AMP, CIP, CL, E, ENR, PF and TA followed by DO (83%), GEN (50%) and N (33%). Sensitivity to Gentamycin was 50% and 33% for N.

All veterinary hospitals *E. coli* isolates were resistant to AMP, E, ENR and PF. CIP was resistant in V1 and V3 samples and sensitive to V2, V4 and V5 samples. Sensitivity to CL was found similarity with CIP. DO were resistant to all isolates except sample number V4, which was intermediate sensitive. Resistance to GEN was lower among isolates that was found only for sample V1 but were Intermediate sensitive to V2 and V3 and were sensitive to V4 and V5. N was sensitive for sample V5 and shown resistance in all other isolates. Sample V5 was intermediate sensitive to TA but V1, V2, V3 and V4 were resistance to TA. Resistance to veterinary hospitals isolates of *E. coli* shown 100% resistance to AMP, E, ENR and PF followed by 80% resistance to DO, N and TA and CL (40%), CIP (40%), GEN (20%). Sensitivity was 40% and 20% for GEN and N, respectively.

*E. coli* isolated from slaughterhouses samples was shown much resistance to tested antibiotics. AMP, CIP, CL, DO, E, ENR, GEN, PF and TA were resistant in all isolates. Antibiotic N was only intermediate sensitive to S3, S4 and S5 but was resistance to S1 and S2. The isolates were shown 100% resistance to AMP, CIP, CL, DO, E, ENR, GEN, PF and TA and 40% to N.

**Table-4.7.a: CS-test for *Salmonella* positive isolates**

|  |  |
| --- | --- |
| **Sample**  | **Antibiotic disc used** |
| **AMP** | **CIP** | **CL** | **DO** | **E** | **ENR** | **GEN** | **N** | **PF** | **TA** |
| **H5** | R | R | S | I | R | R | R | S | R | I |
| **H6** | R | R | S | I | R | R | R | S | R | I |
| **V2** | R | S | S | S | R | R | R | I | R | R |

AMP=Ampicillin, CIP=Ciprofloxacin, CL = Colistin, DO = Doxycycline, E = Erythromycin, ENR=Enrofloxacin, GEN=Gentamycin, N=Neomycin, PF=Pefloxacin, TA= Oxytetracycline R=Resistance I=Intermediate S=Sensitive

**Tabe-4.7.b: Prevalence of hospitals and slaughterhouses *Salmonella* positive isolates**

|  |  |  |  |
| --- | --- | --- | --- |
| **Antibiotic** | **Pattern** | **Medical Hospital** | **Veterinary Hospital** |
| **AMP** | Resistance | 2 (100%) | 1 (100%) |
| Intermediate | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) |
| **CIP** | Resistance | 2 (100%) | 0 (0%) |
| Intermediate | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 1 (100%) |
| **CL** | Resistance | 0 (0%) | 0 (0%) |
| Intermediate | 0 (0%) | 0 (0%) |
| Sensitive | 2 (0%) | 1 (100%) |
| **DO** | Resistance | 0 (0%) | 0 (0%) |
| Intermediate | 2 (100%) | 0 (0%) |
| Sensitive | 0 (0%) | 1 (100%) |
| **E** | Resistance | 2 (100%) | 1 (100%) |
| Intermediate | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) |
| **ENR** | Resistance | 2 (100%) | 1 (100%) |
| Intermediate | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) |
| **GEN** | Resistance | 2 (100%) | 1 (100%) |
| Intermediate | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) |
| **N** | Resistance | 0 (0%) | 0 (0%) |
| Intermediate | 0 (0%) | 1 (100%) |
| Sensitive | 2 (100%) | 0 (0%) |
| **PF** | Resistance | 2 (100%) | 1 (100%) |
| Intermediate | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) |
| **TA** | Resistance | 0 (0%) | 1 (100%) |
| Intermediate | 2 (100%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) |

Out of 16 samples *Salmonella* was found positive in sample number H5, H6 and V2. Resistance to tested antibiotics was found variable among them. AMP, E, ENR, GEN and PF were resistance in all isolates. CL was found sensitive for all sample isolates. DO and TA were intermediate sensitive to H5 and H6 but was resistance to V2. CIP was found sensitive to V2 and resistance to H5 and H6. The level of resistance of *Salmonella* positive isolates were found 100% resistance to AMP, E, ENR, GEN and PF. Lower resistance as CIP (67%), N (67%) and TA (33%) but no resistance was found against DO (0%). Intermediate sensitive was TA (67%), DO (33%) and N (33%) in case of hospitals isolates.

**Table-4.8.a: CS-test for isolates of *Staphylococcus***

|  |  |
| --- | --- |
| Sample  | **Antibiotic disc used** |
| **AMX** | **CH** | **CL** | **CN** | **ENR** | **GEN** | **K** | **N** | **TA** | **P** |
| **H1** | R | R | R | R | R | S | S | R | R | R |
| **H3** | R | R | R | R | R | I | I | R | R | R |
| **H4** | R | R | R | R | R | R | R | S | R | R |
| **H5** | R | R | R | R | R | S | S | S | R | R |
| **H6** | R | R | R | R | I | R | R | S | R | R |
| **V1** | R | R | R | R | R | R | R | R | R | R |
| **V2** | R | R | R | R | S | S | I | S | R | R |
| **S1** | R | R | R | R | R | R | R | S | R | R |
| **S2** | R | R | R | R | R | R | I | R | R | R |
| **S3** | R | R | S | R | R | S | R | S | R | R |
| **S5** | R | R | R | R | S | S | R | S | R | R |

AMX=Amoxicillin, CH=Cefradin, CL=Colistin, CN=Cefalexin, ENR=Enrofloxacin, GEN=Gentamycin, K=Kanamycin, N=Neomycin, TA=Oxytetracycline, P=Penicillin

 R=Resistance I=Intermediate S=Sensitive

**Table-4.8.b**: **Prevalence of hospitals and slaughterhouses isolates *Staphylococcus***

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Antibiotic** | **Pattern** | **Medical Hospital** | **Veterinary Hospital** | **Slaughterhouse** |
| **AMP** | Resistance | 5 (100%) | 2 (100%) | 4 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |
| **CH** | Resistance | 5 (100%) | 2 (100%) | 4 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |
| **CL** | Resistance | 5 (100%) | 2 (100%) | 3 (75%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 1 (25%) |
| **CN** | Resistance | 5 (100%) | 2 (100%) | 4 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |
| **ENR** | Resistance | 4 (80%) | 1 (50%) | 3 (75%) |
| Intermediate | 1 (20%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 1 (50%) | 1 (25%) |
| **GEN** | Resistance | 2 (40%) | 1 (50%) | 2 (50%) |
| Intermediate | 1 (20%) | 0 (0%) | 0 (0%) |
| Sensitive | 2 (40%) | 1 (50%) | 2 (50%) |
| **K** | Resistance | 2 (40%) | 1 (50%) | 3 (75%) |
| Intermediate | 1 (20%) | 1 (50%) | 1 (25%) |
| Sensitive | 2 (40%) | 0 (0%) | 0 (0%) |
| **N** | Resistance | 2 (40%) | 1 (50%) | 1 (25%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 2 (40%) | 1 (50%) | 3 (75%) |
| **TA** | Resistance | 5 (100%) | 2 (100%) | 4 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |
| **PF** | Resistance | 5 (100%) | 2 (100%) | 4 (100%) |
| Intermediate | 0 (0%) | 0 (0%) | 0 (0%) |
| Sensitive | 0 (0%) | 0 (0%) | 0 (0%) |

Ten antibiotics were used from which AMX, CH, CL, CN, TA and PF were resistance to all five medical hospitals isolates. ENR was intermediate sensitive to H6 but shown resistance to rest of the three. GEN and K were sensitive to H1 and H5, intermediate to H3 but were resistance to H4 and H6 isolates. Sensitivity to N was high among other antibiotics, which found sensitive for three isolates as H4, H5 and H6 but was resistance to H1 and H3. The percents of resistance among antibiotics were 100% to AMX, CH, CL, CN, PF and TA followed by ENR (80%), GEN (40%), K (40%) and N (40%). Three of ten were shown sensitivity against the isolates as N (60%), GEN (40%) and K (40%).

Sample number V1 isolates was resistance to all ten tested antibiotics. AMX, CH, CL, CN, OT and PF were resistance to V2, K was intermediate sensitive and ENR, GEN and N were sensitive. 100% resistance was found against AMX, CH, CL, CN, TA and PF. 50 % resistance were found against ENR, GEN, K and N.

All *Staphylococcus* isolates from slaughterhouses were able to shown resistance against AMX, CH, CN, OT and PF. Sensitivity to CL was only found in S3 and was resistance to others. ENR was sensitive to S5 but found resistance to S1, S2 and S3 respectively. S3 and S5 were sensitive to GEN but found resistance against S1 and S2. Intermediate sensitive to K was found for S2 and other isolates were resistance. N was sensitive for three isolates out of four, resistance was only for S2 but found sensitive for S1, S3 and S5. The level of resistance for slaughterhouse isolates *Staphylococcus* to specific antibiotics was: 100% resistances against AMX, CH, CN, TA and PF followed by 75% resistance in CL, ENR and K, 50% to Gentamycin and 25% to N. High level of sensitivity was shown N (75%) and GEN (50%).