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COLONIZATION IN IMMEDIATE POST-MORTEM PULMONARY ASPIRATE: A RATIONAL MEDICO-LEGAL FEATURE IN DIAGNOSIS OF MORTALITY DUE TO VENTILATOR ASSOCIATED PNEUMONIA

Forensic Medicine

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ABSTRACT

Introduction: Diagnosing ventilator associated pneumonia (VAP) is difficult and is usually based on the criteria with high sensitivity and low specificity[1] such as fever, leukocytosis, and infiltrative abnormalities on chest radiographs. In live patients the microbiological analysis of bronchoscopy samples are considered the most specific for diagnosis but not widely used. The Clinical Pulmonary Infection Score (CPIS), a diagnostic algorithm relies on easily available clinical, radiographic, and microbiological criteria. But all these will become hard in cases of medico-legal issue. A study was undertaken to assess the diagnostic value and the impact of microbiological investigations on the accuracy of clinical diagnosis of VAP. This helps to know the microbiological status of lung at the time of death.

Method: A prospective study conducted by qualitative post-mortem culture of pulmonary aspirate taken from each lobe of both lungs among 50 medico-legal cases that were mechanically ventilated.

Results: A sensitivity of 71% and a specificity of 70 was found among the cases where there was inflammatory infiltrations on the chest radiograph and two of three clinical criteria (fever, purulent secretions, leucocytosis). The post-mortem microbiological evaluation corrected the false positives and false negatives.

Conclusion: Conventional clinical criteria have reasonable diagnostic values however Forensic-Microbiology investigations increase the accuracy in diagnosing a medico-legal case of VAP. This can also become a tool for uplifting the justice in such cases. The risk of under treatment and region-wise algorithm guiding antibiotic treatment exclusively by Forensic-Microbiology results improves both the clinical and judicial outcomes.

KEYWORDS

Ventilator associated pneumonia, post-mortem pulmonary aspirate, diagnosis of ventilator associated pneumonia

INTRODUCTION:

"The wards and the post-mortem room show a very striking contrast in their pneumonia statistics". . . Sir William Osler.[2] In the current era of evidence based medicine (EBM), it has become mandatory to provide proper diagnosis for the patient management and/or for the medico-legal purpose after the death. It is important to quote the impact of microbiological testing on the accuracy of clinical diagnosis and post-mortem diagnosis of suspected deaths due to ventilator associated pneumonia (VAP). VAP is a common and fatal nosocomial infection. Diagnosing VAP is difficult and is usually based on the criteria with high sensitivity and low specificity[2] such as fever, leukocytosis, and infiltrative abnormalities on chest radiographs. Deaths due to acute respiratory distress syndrome (ARDS) are adding on to the burden of total mortality and morbidity in developing countries. Clinical criteria for the suspicion of VAP continue to form the salient feature in the diagnosis. It has been mentioned that the diagnosis of VAP should be guided by the quantitative culture results of the bronchoscope retrieved lower respiratory tract samples. In the past, Fàbregas N et al revealed that the clinical criteria have repeatedly been considered as inappropriate for the diagnosis of VAP[3] and on the other hand autopsy studies have concluded that the use of clinical parameters to diagnose VAP leads to over-diagnosis[4] or, even worse, under-diagnosis, particularly in patients with ARDS.[5] A corresponding algorithm would imply that patients with a clinical suspicion of pneumonia but negative microbiological results would not receive antibiotics.[3] The wards and the post-mortem room show a very striking contrast in their pneumonia statistics.[2] Death as investigated by Forensic expert will have significant medico-legal ramifications in addition to providing, valuable information to the medical fraternity, and deceased's family. Thus additional evidence of evaluation of the respiratory distress by the density and the type of microbes causing VAP is essential and the region wise analysis remains important to determine the accuracy of these criteria and management modalities of both medico-legal and non medico-legal cases. Moreover, histological evidence of pneumonia may reflect the clinical stage during evolution but not necessarily the active inflammation responsible for clinical symptoms. It is practically impossible to take biopsy in a case of ARDS for diagnostic purpose but microbiological analysis is possible. We therefore chose to corroborate ante-mortem diagnostic criteria by the presence of positive culture as reference tests for the probable clinically symptomatic VAP and post-mortem diagnosis of VAP, a positive pulmonary aspirate culture.

Methodology:

I. Data collection

After the ethical clearance and consent, 50 apparently normal individuals in the age group 18-60 years with acute respiratory distress following road traffic accidents (RTA), acute poisoning, self-inflicted asphyxia, and snake bites, put on ventilator declared dead due to VAP were studied prospectively. Both sexes will be involved in the study, but we could get only males during the study period. A detailed review of case sheet was conducted and the clinical features were noted along with laboratory parameters like the total count, differential count and chest radiograph. The mean duration of ventilation and type of antibiotic treatment were noted.

ii. Inclusion criteria

Cases clinically diagnosed as VAP on the clinical criteria and CPIS as defined by Johanson et al[6] and Pugin et al[7] respectively whose lungs had been mechanically ventilated for more than 72hours and died in Respiratory Intensive Care Unit (RICU) of major hospitals in Mysore, were considered.

iii. Exclusion criteria

The cases of age less than 18 and more than 60 years were excluded to prevent pediatric and geriatric age related bias. Known patients with primary pathology like immune suppression (Diabetes mellitus, organ transplantation, HIV infection and AIDS, neutropenia, steroid therapy), coronary artery disease and cerebro vascular accidents which add on to the respiratory distress were also excluded.

iv. Sample collection

Immediately after death of the registered medico-legal cases multiple bilateral lung aspirate samples (5 samples/case one from each lobe, right upper, right middle, right lower, left upper, and left lower and from the area of maximal inflammation corresponding to chest

radiographic infiltrates) were obtained for microbiological examination by thoracotomy at the second, fifth and tenth intercostal space in the mid-clavicular line under strict aseptic precaussion before the body was opened completely with the help of sterile disposable syringe with a 20gauge needle. In the absence of inflammation blinded bilateral pulmonary aspirate were taken from upper, middle and lower lobes.

Microbiological Processing and Analysis:
The aspirate was been examined by wet mount microscopy. The inflammatory cells and organisms were identified and tabulated with defining histological and microbiologically active pneumonia. Histological pneumonia was defined on the basis of accumulation of polymorphonuclear leucocytes in the capillaries and adjacent alveolar spaces in at least one sample and microbiologically active pneumonia was defined by the presence of both histological pneumonia and positive lung aspirate cultures.

The samples were inoculated on to brain heart infusion broth, Mac conkey's medium, Chocolate agar and incubated aerobically at 37^0 C for 24 hours. If no growth was found it was further incubated up to 48 hours. The growth was identified by standard protocol by manual phenotypic methods.(Figure 1, 2, 3, 4) The isolates were screened for drug resistance strains like methicillin resistant staphylococcus (MRSA), extended spectrum β lactamases (ESBL), MBL by disk diffusion methods as per CLSI guidelines.

RESULTS:
Total number of cases subjected to study was 50. Samples were collected from all the 5 lobes, right upper, right middle, and right lower, left upper and left lower (Table 1). Among the study population 33 cases (66%) revealed radiographic infiltrations. Sensitivity was high (92%) but specificity was low (33%). The diagnostic accuracy of infiltrates of the right lung was considerably higher than that of the left lung which can be attributed to the anatomic position and more infective foci in right. The general clinical features like fever, cough and breathlessness, leucocytosis, and purulent secretions were predominantly found among 20 (40%), 34 (68%), 40 (80%) and 32 (64%) cases respectively. However there was overlapping of clinical features.

The duration on ventilator (Table 2) were noted. Among the 50 cases 4 were sterile which may be attributed to antibiotic therapy or the obscurity in ante-mortem diagnosis. It will be difficult in such medico-legal cases to prove that death was due to septicaemia and VAP. However these cases revealed radiological infiltration, the finding which may not support the diagnosis fully in the eyes of law and evidence based medicine.

Culture of lung aspirate revealed the growth of Klebsiella, E.coli, Enterobacter, Citrobacter, Proteus, Pseudomonas, Acinetobacter, methicillin-resistant Staphylococcus aureus (MRSA), Streptococcus and Enterococcus. These isolates were belonged to lactose fermenting (NF), non-lactose fermenting (NLF), extended spectrum β-lactamase (ESBM), metalo- β-lactamase, oxidase negative and positive and MRSA. (Table 3) The yield of these cultures showed some similarity with the etiology. (Table 3 and 4) This can be attributed to the institute ecology and will be useful in individual institutional antibiotic policy. The isolates in RTA cases has been summarised in Table 4. Cases with RTA and acute poisoning revealed majority of Klebsiella and Proteus growth along with other bacteria like E coli, Enterobacter, Pseudomonas, Acinetobacter, MRSA and Streptococcus. (Table 4) In RTA and acute poisoning cases a variety of bacterial growth was found when compared with others like snake bite, hanging and drowning. In cases with snake bite we could find Klebsiella, E coli and Proteus; in hanging, Klebsiella and Enterobacter; in drowning only Klebsiella. (Table 3) Apart from these we could able to detect the resistant variety spp, ESBL, MBL. (Table 5)

Among 4 cases (8%) majority of samples did not reveal any growth. The ventilator support in 3 cases was for 2-4 days and cause of death attributed to VAP where as in 1 case it was for 6-8 days and the cause of death attributed to VAP associated with multi organ failure.

DISCUSSION
The dilemma associated with VAP is that, VAP, a common and serious nosocomial infection requires accurate, timely diagnosis and appropriate management. There is still uncertainty in assessing the effect of different empirical antimicrobial therapies on the survival and clinical cure, adverse events, super added infections, length of hospital stay of patients with VAP. It almost doubles the risk of mortality and affects 9-27% of intubated patients.[8, 9, 10, 11, 12] The routine bedside evaluation coupled with radiographic information suggests, but fail to provide definitive evidence that VAP is present or absent. As the severity of respiratory distress increases with ventilation, clinicians should be ready to consider additional tests that provide further evidence for VAP.[13] In today's era of evidence and protocol based management a recognised indisputable method for the validation of diagnostic criteria in diagnosing an ailment is the need of the hour for medico-legal issues. When microbiological results were added to clinical criteria, adequate diagnoses originating from the microbiological investigation which might have corrected false positive and false negative clinical judgements were countered by a similar proportion of inadequate diagnoses.[3]

The diagnosis obtained from clinical parameters like fever, purulent secretions, leukocytosis, and infiltrative abnormalities on chest radiographs will be conditioned with high sensitivity and low specificity. Schurink et al., brings out similar opinion.[14] This inappropriateness can result in delayed administration of appropriate antibiotics which increases mortality, the socio-economic and professional risk factors.[15,16,17]

As a part of work profile it is obvious that the clinical parameters were calculated on the day of death, a fact that does not necessarily correspond with the evolution and/or the pathophysiology features of pneumonia, creating a timing discrepancy between events like onset of pneumonia and death. This is a crucial phase a doctor faces in judicial procedure. Thus this study represents an evaluation of diagnostic criteria in the prevalence of microbiologically active pneumonia on the day of death, on the basis of which the cause of death is made as VAP. However the establishment of pneumonia on the basis of sudden inflammatory response syndrome (SIRS), alveolar haemorrhage, atelectasis, pulmonary infarction, and the fibro-proliferative phase of ARDS should be excluded. This will eliminate the false negatives in cases involved with VAP as the cause of death. It is difficult to establish the diagnosis of pneumonia in the initial phases on the plain chest radiograph. The initial phases of pneumonia in post-mortem histology of lung tissue could be found only 11% of cases.[18] In radio-diagnosis also a technical limitations of chest radiography may hold back the interpretation of radiographs. The radio-diagnostic accuracy may vary in pneumonia due to SIRS, alveolar haemorrhage, atelectasis and pulmonary infarction.

Clinical features like fever, cough and breathlessness, leucocytosis and purulent secretion has a remarkable sensitivity but poor specificity. In resource poor settings, the clinical symptomatology alone cannot be considered as a full proof for diagnosing VAP. Various authors have revealed that fever had 55-100% sensitivity and 20-58% specificity for diagnosing pneumonia in patients with acute respiratory distress syndrome.[19] The vast range of variability depends upon various factors like SIRS, immune-suppression, infections. Leucocytosis and purulent secretion is common in majority of prolonged mechanically ventilated patients which can be attributed to purulent bronchiotis. Rouby[21] et al found that bronchiolitis without pneumonia was not an infrequent finding in mechanically ventilated patients. On the contrary absence of purulent secretion also does not rule out pneumonia which is not uncommon when the foci are in the periphery. Even though the presence of trachea-bronchial secretion is an important clinical feature of VAP, it is not a qualitative finding as the degree of purulence and quantity are highly subjective and biased.

Positive microbiological cultures of tracheal aspirates obtained at the day of the clinical suspicion of VAP, appeared to be the best predictor of VAP. However, these results would only be available 24–48 hours later and this variable is, therefore, hardly suitable for prospective use especially in medico-legal issues. Although surveillance cultures of tracheal aspirates are frequently used for selection of empirical treatment, its benefits on accuracy of empirical therapy in case of VAP have not been determined. The positive predictive value of surveillance cultures to identify the causative microorganism causing VAP was only 18%.[20]

The management of VAP case is highly patient cantered. It depends on features like early treatment based on broad-spectrum antibiotics besieged after direct staining and modified according to microbiological findings, but should never be withdrawn in presence

of negative direct staining or delayed until microbiological results are available. This feature creates confusion among the non-medical people, the judiciary. The association between the clinical features and culture reports in a case of death due to VAP can provide an unbiased finding in judiciary as the level of agreement between different physicians for scoring individual variables varied greatly. The absence of a true gold standard for VAP, and medicine being a science of probability hampers the interpretation of any study investigating the diagnostic approach of this infection. We have preferred to use qualitative cultures of pulmonary aspirate in post-mortem examination to correlate clinical features and to assist in judiciary.

The vast variation in culture among cases with different etiology can be attributed to the limited exposure of cases for both environment and ventilator. (Table 3) The severity of VAP is directly proportional to the duration of ventilator support and exposure to different environment. This is more severe when it is associated with head injury where cardio-respiratory functions were affected due to involvement of mid-brain. In a patient-based approach study it has been revealed that methicillin-sensitive Staphylococcus aureus (MSSA) should be expected in comatose patients and methicillin-resistant Staphylococcus aureus (MRSA) should not be expected in patients without previous antibiotic coverage.[21] However in the present study MSSA obtained from altered conscious and also from comatosed patients and MRSA was found in patients without previous antibiotic coverage. Pseudomonas aeruginosa should be covered with combination therapy.[21] Vancomycin, given at the standard doses and route of administration for the treatment of VAP caused by Gram-positive pathogens, is associated with poor outcomes. The choice of initial antibiotic should be based on the patient's previous antibiotic exposure and comorbidities, and local antibiotic susceptibility patterns, which should be updated regularly.[21] But in our set it is difficult to get the history of patient's previous antibiotic exposure however we have started to implement the concept of local antibiotic susceptibility patterns and infection control team at the tertiary care level. Majority of professionals accept that optimal management of clinically suspected VAP remains open to debate.

In the present study majority of cases revealed the presence of infection with ESBL-producing pathogens, particularly Klebsiella pneumoniae. Infections caused by multidrug-resistant Gram negative bacilli that produce ESBL enzymes have been reported with increasing frequency in intensive-care units and are associated with significant morbidity and mortality. This demands periodic microbiological surveillance of critical wards where multi drug resistant bugs are present and depending on the report antibiotic policy has to be introduced.

Among the cases no differences were found in the mortality and/or morbidity between mono therapy and combination therapy. We determine that clinical features for this comparison to be low quality evidence whereas cause of mortality, and length of ICU stay as moderate quality evidence. In majority of cases II and III generation cephalosporins were used from the day of admission and in prolonged ventilator usage cases meropinum, imipenem-cilastatin, β-lactamase inhibitors like tazobactum which was added to extend the β-lactum antibiotic piperacillin. Drugs like tazobactam and clavulanic acid have been shown to inhibit ESBL enzymes. Even though the combination of tazobactam-piperacillin, has broad-spectrum antimicrobial activity against a wide variety of bacteria including ESBL-producing bacteria is effective –in bloodstream infections, urinary tract infections, therapeutic failures have been documented abscess presumably containing a large number of organisms. Other group, the carbapenems, imipenem and meropenem, have consistently been effective against ESBL-producing Klebsiella and the nano sized carbapenem particles access easy way through porins into Gram negative bacilli.

It is evident that there is no difference between mono and multi therapy approach in VAP management. This data cannot be generalised as the study rules out the consideration of antibiotic resistance of the organisms cultured, however it is an alarming signal for the hospitals where the cases were treated and to form a protocol management involving frequent culture and sensitivity of the nosocomial organisms. It is difficult to fix the best antibiotic in VAP management.[22]

CONCLUSION

Fever, leucocytosis, purulent pulmonary secretions along with radiological infiltration do not substantively alter the probability of VAP. However, the combination of a new radiological infiltration with frequent episodes of fever, leucocytosis, or copious purulent sputum associated with subsequent tachypnea, tachycardia, hypoxemia increases the likelihood of VAP. The absence of a new infiltrate on a plain chest radiograph lowers the likelihood of VAP. Fewer than 50% neutrophils on cell count analysis of lower pulmonary secretions make VAP unlikely. The choice of initial antibiotic should be based on the patient's previous antibiotic exposure and comorbidities, and local antibiotic susceptibility patterns, which should be updated regularly by infection control team and the antibiotic policy of the institute. In this clinical scenario it will be difficult for the Forensic Expert to diagnose VAP as the cause of death without post-mortem evidence and Microbiologist intervention. Thus a through Forensic-Microbiology assay and clinical correlation removes the hurdles in expert evidence.

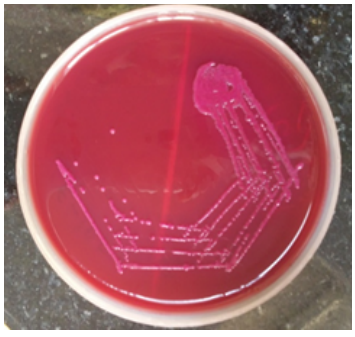

Figure 1: MacConkey agar with Lactose fermenter and Non Lactose Fermenter colony

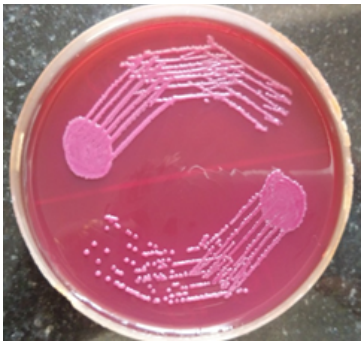

Figure 2: Culture of Klebsiella pneumoniae on MacConkey agar

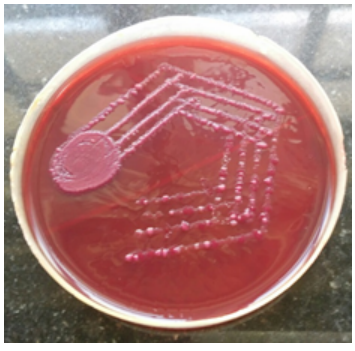

Figure 3: Culture of pseudomonas and E coli on MacConkey agar

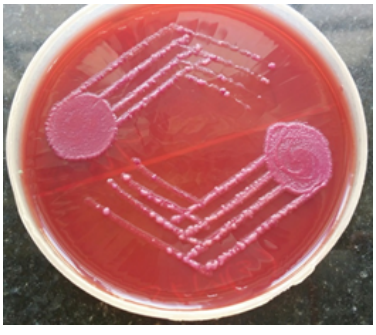

Figure 4: E coli colony morphology on MacConkey agar plate

LUNG SAMPLES STUDY

Table 1: Distribution of cases and total samples cultured on the basis of etiology

History	Cases	Samples Cultured (5 samples from each case)	Percentage (%)
RTA	26	26X5=130	52
Acute OP poisoning	12	12X5=60	24
Snake bite	4	4X5=20	8
Hanging	4	4X5=20	8
Rat poisoning	2	2X5=10	4
Drowning	2	2X5=10	4
TOTAL	**50**	**50X5=250**	**100**

Table 2: Distribution of cases on the basis of duration on ventilator

DURATION ON VENTILATOR (DAYS)	NUMBER	PERCENTAGE (%)
2 – 4	40	83
4 – 6	6	13
6 – 8	4	4

Table 3: Distribution of organisms cultured from the lobes

Case	RU lobe	RM lobe	RL lobe	LU lobe	LL lobe
Snake bite cases					
1	Non ESBL, LF-Klebsiella	Non ESBL, LF-Klebsiella	Non ESBL, LF-Klebsiella	Non ESBL, LF-Klebsiella	Non ESBL, LF-Klebsiella
2	Non ESBL, LF-Klebsiella	-	Non ESBL, LF-Klebsiella	Lf-Klebsiella Pseudomonas	Lf-Klebsiella Pseudomonas
3	ESBL, LF-Klebsiella	LF-Klebsiella	-	-	-
4	ESBL, LF-Klebsiella	LF-Klebsiella	ESBL, LF-Klebsiella	LF-Klebsiella	-
Acute poisoning cases					
5	NLF-Klebsiella Staphylococcus Acinetobacter	Staphylococcus	LF-Klebsiella ESBL-E coli	LF/NLF-Klebsiella Acinetobacter Pseudomonas	LF/NLF/ESBL Klebsiella
6	Enterococcus Staphylococcus (Catalase –ve)	-	LF-Klebsiella	-	-
7	LF/NLF-Klebsiella	LF/NLF-Klebsiella	LF/NLF-Klebsiella	LF/NLF-Klebsiella	LF/NLF-Klebsiella
8	ESBL/MBL Klebsiella	ESBL/MBL Klebsiella	- MSSA	ESBL/MBL Klebsiella, MSSA	-
9	-	-	-	-	-
10	-	-	-	-	-
11	NLF-Klebsiella LF (mucoid)-Klebsiella	NLF-Klebsiella ESBL-Enterobacter	-	-	-
12	NLF-Klebsiella LF (mucoid)-Klebsiella	NLF-Klebsiella ESBL-Enterobacter Citrobacter	NLF-Klebsiella	NLF-Klebsiella	NLF-Klebsiella
13	ESBL/MBL Klebsiella	MRSA	MRSA	ESBL/MBL Klebsiella	-
14	-	-	-	-	-
15	NLF-Klebsiella Acinetobacter	-	LF-Klebsiella	LF/NLF-Klebsiella Acinetobacter	LF/NLF/ESBL Klebsiella
16	LF/NLF-Klebsiella MSSA	LF/NLF-Klebsiella MSSA	LF/NLF-Klebsiella	NLF-Klebsiella	NLF-Klebsiella
17	NLF-Klebsiella LF (mucoid)-Klebsiella	NLF-Klebsiella ESBL-Enterobacter	NLF-Klebsiella	NLF-Klebsiella	NLF-Klebsiella
18	NLF-Klebsiella LF (mucoid)-Klebsiella	NLF-Klebsiella ESBL-Enterobacter	NLF-Klebsiella	NLF-Klebsiella	NLF-Klebsiella
Hanging-Asphyxia cases					
19	LF-Klebsiella	LF-Klebsiella	LF-Klebsiella	LF-Klebsiella	LF-Klebsiella
20	-	-	Pale LF Oxidase negative Enterobacter	Pale LF Oxidase negative Enterobacter	-
21	LF-Klebsiella	LF-Klebsiella	LF-Klebsiella	-	-
22	LF-Klebsiella, LF Oxidase negative Enterobacter	LF-Klebsiella	-	LF Oxidase negative Enterobacter	-
Drowning-Asphyxia cases					
23	MA LF-Klebsiella PLF- Klebsiella	MA LF-Klebsiella PLF- Klebsiella	MA LF-Klebsiella	MA LF-Klebsiella	-
24	LF-Klebsiella, LF Oxidase negative Enterobacter	LF-Klebsiella	LF-Klebsiella	LF-Klebsiella	-

(Isolates in RTA Cases has been summarised in Table 4)

Table 4: Isolates tabulated as per the etiology

Organisms	RTA (n=26)	Acute poisoning (n=14)		Snake bite (n=4)	Hanging (n=4)	Drowning (n=2)
		Rat poisoning	OP poisoning			
Klebsiella	+	+	+	+	+	+
E.coli	-	-	+	+	-	-
Enterobacter	+	-	+	+	+	-
Citrobacter	-	+	+	-	-	-
Proteus	+	-	-	+	-	-
Pseudomonas	+	-	+	-	-	-
Acinetobacter	+	-	+	-	-	-
MRSA	+	-	+	-	-	-
Streptococcus	+	-	-	-	-	-
Enterococcus	-	-	-	-	+	-

+ Growth present
-No growth

Table 5: Resistant isolates from the samples

ORGANISMS	NUMBER	SPP	ESBL	MBL	ESBL+MBL
Klebsiella	116(46.4%)	40(34.48%)	4(3.44%)	4(3.44%)	8(6.89%)

E.coli	16(6.4%)	8(50%)	8(50%)	-	-
Enterobacter	40(16%)	24(60%)	16(40%)	-	-
Citrobacter	2(0.8%)	-	-	-	-
Proteus	12(4.8%)	4(33.33%)	4(33.33%)	-	4(33.3%)
Pseudomonas	8(3.2%)	-	-	-	-
Acinetobacter	2(0.8%)	-	-	-	-
MRSA	20(8%)	-	-	-	-
Streptococcus	8(3.2%)	-	-	-	-
Enterococcus	6(2.4%)	-	-	-	-
No growth	20(8%)	-	-	-	-
TOTAL	250				

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