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Utility of Chromogenic Agar Media for Isolation and Presumptive Identification of Urinary Bacterial Pathogen and Analysis of Antibiotic Sensitivity Pattern

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A B S T R A C T

Urine samples are the most common specimens sent to the microbiology laboratory. Rapid identification of the pathogens provides useful information to the clinician for initiating appropriate antibiotic therapy. Most of the laboratories use blood agar, MacConkey agar for primary isolation of urine samples. HiCrome UTI agar is such a versatile primary culture tool that facilitates rapid isolation as well as presumptive identification and differentiation of most uropathogens. The Microbiological performance of HiCrome UTI agar medium was compared With Blood agar and MacConkey agar for isolation and presumptive identification of Bacteria from urine culture and to determine the antibiotic sensitivity pattern of isolates obtained. Also check the multidrug resistance pattern of obtained isolates. From the microbiology laboratory urine sample collected and will be inoculated on Bloodagar, MacConkeyAgar, CHROME agar. Organism identified and compared with conventional method and then antibiotic sensitivity test will be done according to CLSI guidelines. Also check their multidrug resistance pattern. Out of 874 urine culture, 481 yielded bacterial growths and 393 showed no growth. Gram negative organisms predominate showing. Urinary tract infection was more prevalent in age group between 21-40 years. E. coli is most predominant bacteria isolated (150). The ability of Hicrome UTI agar to detect Uropathogens is equal to that of the combination of the two reference media Blood agar and MacConkey agar. All the 429 were identified in HiCrome UTI agar (100%) whereas From Blood agar and MacConkey agar (99%) and (93%) respectively. In case of presumptive identification HiCrome UTI agar precedes the other conventional media. Out of 68 mixed bacterial growths all (100%) were detected by HiCrome UTI Agar on second day itself. Most of the isolates are showing high resistance to antibiotics. In conclusion HiCrome UTI agar is recommended as single medium for direct isolation, presumptive identification. The Higher prevalence of urinary tract infection was seen in females than males. E. coli was the predominant pathogen responsible for Urinary tract infection followed by Klebsiella pneumoniae. The present study observed the high prevalence of multidrug resistance among bacterial uropathogens. Particularly, rate of resistance to carbapenems and third generation cephalosporins were higher. Early detection and close monitoring of MDR must be started by all clinical microbiology laboratories to reduce the antimicrobial resistance that is now a global problem.

Introduction

Urinary tract infection is one of the most prevalent bacterial infections in developing countries. A bacterial infectious process affecting any part of urinary tract most commonly in bladder and urethra. Among the hospital acquired infection UTI contribute 30-40% of them. It has a spectrum of clinical entities with severity ranging from asymptomatic infection to acute pyelonephritis with sepsis. Although many of these infections are treated empirically. They also cause morbidity and increased mortality in hospitalized patients. [1] UTI caused by bacteria that can also live in digestive tract, in vagina, or around the urethra most often these bacteria enter the urethra and travel to bladder and kidney and prostate (men).

The prevalence of UTI is age and sex dependent with more of sex dependent towards females. The anatomy of female urethra is of particular importance to pathogenesis of UTIs. The anatomic structure of female urethra is too short so it is close proximity to the perianal region, bacteria can reach bladder more readily and cause infection. Hormonal changes during pregnancy can also leads to UTIs. Other predisposing factors like sexually active women tend to have more UTI than do women who are not active, diabetes, urinary calculi, structural and functional abnormalities of urinary tract, Renal disease and indwelling urinary catheters. Infection occur by two major routes, that one is ascending route which is the most common route of spread of infections whereas descending route by hematogenous spread occurs as a result of bacteremia.

Urine samples are the most common specimens sent to the microbiology laboratory. Rapid identification of the pathogens provides useful information to the clinician for initiating appropriate antibiotic therapy. Diagnosis of urinary tract infections contributes significantly to the daily work load in microbiology laboratory. Culture media have an essential role in the field of diagnosis which helps in the easy and reliable detection of all urinary pathogens.

Therefore any medium with ability to show results within a short time i. e. reduced turnaround time (TAT) and laboratory cost is used in many microbiology laboratories. Most of the laboratories use blood agar, MacConkey agar or Cysteine Lactose Electrolyte Deficient Agar (CLED) for primary isolation of urine samples. Though blood agar is used as an enriched medium it lacks the ability to primarily differentiate between Gram positive and Gram negative organisms, which needs further identification tests and delay in result. Swarming of Proteus species is not prevented in blood agar. Lactose fermenting and non-lactose fermenting organisms are differentiated by MacConkey agar using neutral red indicator. [2]

Hence Blood agar and MacConkey agar medium are used as a conventional media for the isolation of urinary pathogens. MacConkey agar and CLED agar media helps to distinguishes Lactose fermenting and non-lactose fermenting colonies and also prevent swarming of Proteus spp. But further differentiation of organisms are not possible because these media lacks genus or species specific indicators so subculture or biochemical tests is necessary for further identification which leads to longer reporting time and cost.

Now a day's CHROMOGENIC agar (CA) media as an alternative method to overcome these limitations. These media help in identification of pathogen on the primary plate itself by the typical color and morphology produced by the organisms there by reducing confirmatory tests. [3] HiCrome UTI agar is such a versatile primary culture tool that facilitates rapid isolation as well as presumptive identification and differentiation of most uropathogens including various species from mixed cultures in clinical urine specimens. The media containing Chromogenic substrates that are broken down by bacterial enzymes from clinical specimen imparting a unique visible color to the growing bacterial colonies for their identification. [4, 5] This single medium supports the growth of all uropathogens and supports the mixed growth infections also, so it can be diagnosed more easily.

In a few studies comparing chromogenic media with conventional methods its advantages including reduction in time for identification, workload, rapid recognition of mixed growth without any doubt and reduction in number of many biochemical tests for bacterial identification have been used. All these factors which lead to total cost reduction. [6, 7]

Antimicrobial resistance is a global public health treat. In the majority of the cases antimicrobial treatment is usually started empirically before the laboratory results of urine culture are available. This antimicrobial resistance may increase in uropathogen because of the frequent and improper use of antibiotics. Knowledge of antimicrobial resistance pattern of common uropathogen

is essential to provide clinically appropriate and cost effective therapy and reduction in antimicrobial resistance. The sensitivity of bacterial urine isolate to commonly used antimicrobial agent from different group was determined using standard disc diffusion method following clinical and laboratory standards institute guidelines

UTI due to multidrug resistance bacteria have been rise globally with serious complication for public health. Resistance to at least one agent in 3 or more antimicrobial categories defined as MDR uropathogen especially ESBL producing bacteria that have been rise globally. MDR uropathogen has now a universal problem and more alarming in developing countries.

Materials and Methods

Clinical syndrome-based urine sample were collected.

Microscopic examination of urine

Wet film and gram stain will be done for the urine samples.

Culture

Urine samples inoculated on blood agar, MacConkey agar, CHROME agar. The plates will be incubated at 37c overnight. Presumptive identification of bacteria on the MacConkey agar, blood agar, CHROME agar was done according to the colony morphology and colour. Find out which media helps in the early detection of pathogen.

Antibiotic sensitivity testing of isolates obtained from clinical samples was carried out by Kirby-Bauer disc diffusion method.

Results and Discussions

In the present study, a total of 874 urine samples collected from clinically suspected UTI cases from outpatients and inpatients during the study period. Out of 874 urine culture, 481(55%) yielded bacterial growths and 393 (44.9%) showed no growth.

Urine culture results among study population

Among the 481 samples which were Culture positive, 413 of them showed a single isolate (85.8%). 34 (14.1%) of the urine samples showed more than one type of organisms. if more than ≥100 colonies are counted for at least one of the two types was considered as mixed growth.

Type of growth among positive isolate

Out of 481 urinary isolates, Gram negative organisms predominate showing 348(72.3%).

The Gram positive organisms and yeasts were isolated at a rate of 81(16.8%) and 52(10.8%) respectively.

Distribution of culture positive isolates

In this study, Urinary tract infection was more prevalent in age group between 21-40 years (42.6%) followed by 41-60years (32.4%) and least common in age group is >60 (12.5%) years and highest incidence in females (66.9%) and rest (33.5%) in males.

Age wise distribution of patient population

Gender wise distribution of patient population

In urinary tract infection, 348 Gram negative isolates were isolated, 150 (31.1%) of them were *Escherichia coli* followed by *Klebsiella pneumoniae* 97(20.1%), *Pseudomonas aeruginosa* 36 (7.4%), *Enterobacter* spp 27(5. 6%), *Acinetobacter spp* 13(2.7%), *Citrobacter spp* 10 (2.0%), *Proteus spp* 9(1.8%) and *Serratia spp* 6(1.2%).

Among 81 (16.8%) Gram positive isolates isolated, 51(10.6%) of them were *Enterococcus faecalis* followed by *Staphylococcus aureus* 21(4.3%), *Streptococcus agalactiae* 9(1.8%). Other isolates was yeasts 52(10.8%).

Distribution of organisms isolated

The ability of HiCrome UTI agar to detect uropathogens is equal to that of the combination of the two reference media Blood agar and MacConkey agar.

All the 429 were identified in HiCrome UTI agar (100%) whereas From Blood agar and MacConkey agar 426(99%) and 401(93%) respectively.

In MacConkey agar all other organism showed 100% isolation rate Except *Enterococcus* faecalis 26(50.9%).

Comparison of three culture media for rate of isolation

In case of presumptive identification HiCrome UTI agar precedes the other conventional media. Highest presumptive identification rate 422(98%) was found in primary culture plates of HiCrome Agar media followed by 348 (81%) in Blood Agar and lowest rate 319(74%) was found in MacConkey Agar.

Rate of presumptive identification

Out of 68 mixed bacterial growths all (100%) were detected by HiCrome UTI Agar on second day itself; 94% by Blood agar and 44. 1% by MaConkey agar. Detection of mixed bacterial growth is easier in HiCrome UTI Agar based on color.

Organisms isolated from mixed growth

Susceptibility pattern of gram negative isolates

Antibiogram of *E. coli*

When the antibiogram pattern of E. coli isolates to different drugs are studied, E. coli showed maximum sensitivity to Fosfomycin (96%) followed by Nitrofurantoin (92%), Imipenem (88%), Meropenem (87%), Cefipime Tazobactam (86%), Piperacillin Tazobactam (85%), Cefoperazone Sulbactam (84%), Amikacin (76%), Gentamicin (74%), Aztreonam (58%), Cefipime (54%), Cotrimoxazole (54%) and least sensitive to Ceftriaxone (42%).

Maximum resistance was seen with Ampicillin (100%) followed by Ceftriaxone (56%), Cefipime (46%), Ciprofloxacin (44%), Doxycycline (39%). Among 150 isolates of E. coli 48 (32%) was ESBL positive isolates and 18 (12%) was carbapenem resistant Isolates (CRE). All the 18 carbapenem resistant isolates were found to have intermediate susceptibility to Colistin.

Antibiogram of Klebsiella pneumoniae

When the antibiogram pattern of *Klebsiella* pneumonia isolates to different drugs were studied, it shows maximum sensitivity to Minocycline (65.9%) followed by Meropenem (57%), Cefipime Tazobactam (56%) and least sensitive to Nitrofurantoin (26. 8%). Maximum resistance was seen in Ampicillin (100%), followed by Ceftriaxone (65%), Nitrofurantoin (62.8%), Aztreonam (60. 8%), Cefipime (60.8%), Cotrimoxazole (58.7%). Among 97 isolates of *Klebsiella* pneumonia 17(17.5%) were ESBL positive isolates and 42(43.2%) were carbapenem resistant (CRE) isolates.

Antibiogram of Pseudomonas aeruginosa

When Antibiogram of Pseudomonas aeruginosa isolates to different drugs were studied, it shows maximum sensitivity to Ceftazidime (72.2%) followed by Aztreonam (72%), Imipenem (61%), Amikacin (61%). Maximum resistance was seen in Nitrofurantoin (100%). Among 36 isolates of Pseudomonas aeruginosa 14(38.8%) were Carbapenem resistant isolates.

Antibiogram of Enterobacter spp

When Antibiogram of Enterobacter isolates to different drugs were studied, it shows Maximum sensitive to Meropenem (92%), followed by Levofloxacin (96%) and Maximum resistance to Nitrofurantoin (44.5%). Among 27 isolates one isolates each were ESBL and CRE (3.7%).

Antibiogram of Acinetobacter spp

When antibiogram of Acinetobacter isolates to different drugs were studied, it shows maximum sensitive to Cefaperazone sulbactam, Cefipime Tazobactam (100%). Maximum resistance to Nitrofurantion (92.3%). Among 13 isolates 2 (15.3%) were Carbapenem resistant Acinetobacter.

Antibiogram of Citrobacter spp

When Antibiogram of Citrobacter isolates to different drug were studied, it shows maximum sensitive to almost all drugs Cefipime, Ceftriaxone, Imipenem etc. (100%).

Antibiogram of Proteus

When antibiogram of Proteus isolates to different drug were studied, it shows maximum sensitive to Meropenem, Imipenem, Amikacin (100%) Maximum resistance was seen in Nitrofurantoin (100%).

When antibiogram of Serratia spp isolates to different drugs were studied, it shows maximum sensitive to Imipenem, Meropenem, and Cotrimoxazole (100%). It

shows High resistance to Nitrofurantoin (100%). Ampicillin and Colistin was not reported for Serratia due to intrinsic resistance.

Note: All Carbapenem resistant gram negative isolates Colistin MIC was checked. All isolates showing intermediate to Colistin.

Susceptibility pattern of gram positive isolates

Antibiogram of Staphylococcus aureus

When antibiogram Pattern of Staphylococcus aureus was studied, they show maximum sensitive to Minocycline, Nitrofurantoin, and Linezolid (100%). It shows High resistance to Ciprofloxacin (52%), Cefoxitin (33%). Among 21 isolates of Staphylococcus aureus, 7 (33.3%) were MRSA isolates.

Antibiogram of *Enterococcus* spp

When antibiogram pattern of *Enterococcus* faecalis was studied, it shows Maximum sensitive to Linezolid (100%) followed by Vancomycin and Teicoplanin (100%). It shows High resistance to Tetracyclin (76%) followed by HLG (50.9%). Among 51 isolates of *Enterococcus* faecalis, No (0%) VRE positive Isolates.

Antibiogram of Streptococcus agalactiae

When antibiogram pattern of Streptococcus agalactiae was studied, it shows maximum sensitive to almost all drugs (100%). Maximum resistance was seen in Cotrimaxasole (100%).

Out of 481 urine isolates, 150(31.1%) was MDR isolates. Among them 77(51.3%) were CRE positive isolates, 66(44%) ESBL positive isolates and 7(4.6%) were MRSA isolates. Among the 66 ESBL positive isolates Escherichia coli 48(72.7%) was the predominant ESBL producing organism followed by *Klebsiella* pneumonia 17 (25.7%). Among the 77 CRE positive isolates *Klebsiella* pneumonia 42(54.5%) was predominant CRE isolate followed by E. coli 18 (23. 3%).

The standard diagnosis of UTIs is the culture of urine sample on solid media. . Blood agar and MacConkey agar are the conventional media used in our laboratory. The present study evaluated the ability of Blood agar, MacConkey agar, HiCrome UTI agar for detection and identification routine uropathogens. Previous studies have demonstrated equal or superior performance of chromogenic media compared to conventional media for isolation and identification of uropathogens. The results from this study were in agreement with prior reports.

In the present study, out of 874 urine samples, 481 (55%) urine sample showed positive growth and remaining samples 393(44.9%) had no growth. Of them 413(85.80%) samples showed single organism and 68 (14.10%) showed mixed growth. This was slightly higher the study done by Leeela rani *et al.,* [8], which revealed 95. 8% were monomicrobial and 4.12% were polymicrobial growth. The study done by Lakshmi *et al.,* [9], obtained 95.12% monomicrobial and 4. 87% polymicrobial growth. Other study by Soley Sharmin *et al.,* [10] showed 90.6% Monomicrobial and 9.4% polymicrobial growth which was slightly similar to our study. This study had slight difference with the study done by R Praveen *et al.,* [11] which showed 31.67% Monomicrobial and 2% polymicrobial growth.

In the present study Gram negative organism were responsible for 72.3% of UTIs and Gram positive organism were responsible for 16.8% of UTIs and remaining 10.8% were caused by yeast. The studies by Arwa M Abdullah *et al.,* [12] showed 75.33% of UTI were caused by Gram negative and 24.66% by Gram positive bacteria. Another study by Z. Samra *et al.,* [13] showed 70% of Gram negative organisms, 26% Gram positive organisms and 4% of Candida species were responsible for UTI s. The present study showed slight decrease in isolation of Gram positive organisms, but greater recovery of yeasts isolates.

In our study more pathogens were isolated from females (66.9%) than males (33.5%). This was in correlation with the study conducted by Sabrina J Moyo *et al.,* [14], in which the isolation of pathogens from females were at a rate of 54.4% and from males were at a rate of 45.6%. Another study by B. Sasirekha *et al.,* [15] also showed that females (51.3%) were affected more commonly than males (48.6%).

In our study highest incidence of urinary isolates were from 21-40 (42.6%) years age group followed by 41-60 (32.4%) years. This correlates with studies done by Anbumani N *et al.,* [16]. Also this was in consistent with a study by Beyene G *et al.,* in which 53.5% were in the age group between 19-39 years. This is in coincident with the study by S. Shafiyabi *et al.,* [17] in this study here is a

peak incidence of UTI was noted in the age group between 21-50 years. (55%).

Escherichia. coli is the most common isolated organism (31.1%) in our study followed by *Klebsiella* pneumoniae (20.1%) among gram negative uropathogens which is consistent with many other studies by Razak SK *et al.,* Sibi *et al.,* [18, 19]. According to this study E. coli were isolated (37. 95%) and *Klebsiella* pneumonia were isolated (21.41%). When correlate with another study done by Chaudhary Navin kumar *et al.,* [20] which showed higer rate 52.4% of E. coli and another study by Sohely sharmin *et al.,* [10], which showed 53.2% of E. coli [10] Among gram positive isolates *Enterococcus* Faecalis is the most common isolated organism (10. 6%) followed by Staphylococcus aureus (4.3%), which is consistent with study done by Das RN *et al.,* [21], isolation rate of Enterococus is 8.2%. Another study by Sohely Sharmin *et al.,* [10], the isolation rate of *Enterococcus* was 10.7%.

In our study the isolation of organisms from Hicrome UTI agar (100%), (99%) in Blood agar and (93%) in MacConkey agar, showed mostly similar isolation rates as that of the study by Leela Rani *et al.,* [8], (Blood agar (97. 5%), MacConkey agar (89.1%) and HiCrome UTI Agar (100%)). Blood agar is an enriched medium and HiCrome UTI agar also contains all essential nutrients to support the growth of possible uropathogens that is why all isolates were possible to be grown on to these two media and similar findings were also reported by Parveen *et al.,* [11], *Enterococcus* produced characteristic tiny blue appearance. This was more easily identified by Chromogenic agar. Due to the characteristic color and morphology they can be easily identified from mixed cultures. *Klebsiella*, Enterobacter and Citrobacter Produced similar colony colors which were further identified by Biochemical reactions. This was also observed in a similar study by Lakshmi *et al.,* [4].

In case of presumptive identification Hicrome UTI Agar precedes the other conventional media. Presumptive identification of bacterial isolates in urine culture is time consuming and requires a great deal of experience when using traditional media like Blood agar, MacConkey agar. In our study on Hichrome UTI agar 98% of the isolates presumptively identified while BA and MA in combination could presumptively identify only 81% and 74% respectively. These results are somewhat similar with the results noted by R Perveen *et al.,* [11], According to this study 94. 39% of isolates were presumptively identified by HiCrome UTI agar media and 77.57% by Blood agar and MacConkey agar media in combination. On the other hand, HiCrome UTI agar presumptively identified 97. 49% of isolates whereas MA and BA contributed in 67.34% and 36.68% presumptively identification of organisms, respectively, according to the study by Laila Akter [22].

Rafaat *et al.,* [23], showed that the isolation and identification of isolates was found to be best on chromogenic UTI agar (98.9%) and least favorable by the conventional method (94.4%). The present study revealed that, 100% E. coli were presumptively identified on Hicrome UTI agar whereas 77% and 94% E. coli were presumptively identified in BA and MA, respectively. These results are consistent with the study by R Parveen *et al.,* [11], which stated 94.20% Escherichia coli, were identified in HiCrome UTI agar, whereas 79.71% were in CLED agar media and 82.61% in MacConkey & Blood agar media.

The present study also reveals that Hicrome UTI agar contributed 100% presumptively identification of *Klebsiella* spp. whereas BA could identify 97% of *Klebsiella* isolates. These results are very similar with the results in a study by Laila Akter [22], which stated 100% *Klebsiella* isolates were presumptively identified on Hicrome UTI agar. In our study *Enterococcus* were presumptively identified on Hicrome agar (100%) and none of the *Enterococcus* was presumptively identified on BA and MA in combination. A study sharmin *et al.,* [10], by in Bangladesh revealed that 100% enterococci were identified on HiCrome UTI agar. According to R Preveen *et al.,* [11], 100% were identified on Hicrome agar media.

In the present study isolation of organism from mixed culture were 100% in HiCrome UTI agar followed by 94% in Blood agar and 44% in MacConkey agar. This was similar to the study done by R Parveen *et al.,* [11], and Leela Rani *et al.,* [8], which also showed similar results. This shows 100% on HiCrome UTI agar. HiChrome UTI agar media offer a far superior means of differentiating polymicrobial cultures from pure cultures, thus enabling microbiologists to assess more accurately the clinical relevance of urine culture results. Improved detection of mixed cultures may help to identify contaminated specimens and therefore lead to a reduction in the prescription of unnecessary antibiotics. Similar to our study, studies by Leela Rani *et al.,* [8], Lakshmi *et al.,* [4], and J D Perry *et al.,* [3], observed easy identification of organisms from mixed cultures in Chromogenic agar and

also in identifying contaminated samples.

Since the presumptive identification of the common organisms Were possible in the primary plate itself, it was observed that the Turnaround time of reporting the urine culture could be reduced. Chromogenic media though more expensive, can be Cost effective due to easier recognition of significant isolates from Culture plates and more accurate detection of mixed cultures. The study by Retelj and Harlander[24], have concluded that chromogenic Media for urinary tract pathogens is cheaper than conventional Methods only when there is high rate of isolation of organisms Such as Escherichia coli, Enterococci *Klebsiella* and Proteus. Other researchers; Samra *et al.,* [25], and engstler *et al.,* [26], speculated that the speed and reliability of identification on chromogenic media may render them cost effective, although the price of chromogenic media is much higher than traditional media.

The chromogenic media can be used as a single primary isolation media for identification of urinary tract pathogens. Several researchers - Aspell *et al.,* [27], Carricajo *et al.,*[28], and Scarparo *et al.,*[29], have previously demonstrated equal or superior performance of various chromogenic media over traditional media for identification of urinary tract pathogens.

In the antibiogram pattern of E. coli isolates to different drugs E. coli shows maximum sensitivity to Fosfomycin (96%) followed by Nitrofurantoin (92%), Imipenem (88%), Merpenem (87%), Piperacillin Tazobactam (85%), Cefaperazone Sulbactam (84%) Amikacin (76%) and Gentamicin (74%). Maximum Resistance was seen with Ceftriaxone (56%), Cotrimoxazole (46%), Cefipime (46%), Ciprofloxacin (44%). which was similar to the other studies of Kothari A *et al.,* [30] and Colodner R *et al.,*[31], Nitrofurantoin, which was found to be effective Against sensitive against E. coli isolates. This more resembles with the other studies of Nicolle and Amdekar *et al.,* [32].

In the antibiogram pattern of *Klebsiella* pneumonia isolates to different drugs, *Klebsiella* shows maximum sensitivity to amikacin (56%) followed by Cefipime Tazobactam (56%), Meropenem (57%) and Maximum Resistance to Ceftriaxone (65%) followed by Nitrofurantoin (62%), Cefipime (60%). Which was similar to the studies by Maheswary *et al.,* [33], Khan *et al.,* [34], Dash *et al.,* [35]. According to their studies most isolates are low sensitivity to third generation Cephalosporins?

In the antibiogram pattern of *Enterococcus* isolates showing Maximum sensitive to vancomycin (100%) followed by Linezolid (100%), Teicoplanin (98%), Nitrofurantoin (72%). This was similar to other studies Haritsa *et al.,* [36], Barman *et al.,* [37], shanmukappa *et al.,* [38], sreeja *et al.,* [39], Bhakare *et al.,* [40]. According to their studies almost all isolates in this study were sensitive to Linezolid & Teicoplanin.

In our study 72.7% Escherichia coli and 25.7% *Klebsiella* pneumoniae isolates were found to be Extended Spectrum Beta Lactamases (ESBL) producers. This is much lower as compared to study done by Shobha KL *et al.,* [41], where 35% Escherichia coli and 41% *Klebsiella* pneumoniae were found to ESBL producers and this is much higher as compared to study done by Mohammed A *et al.,* [42], study 34. 4% Escherichia coli and 27.3% *Klebsiella* pneumoniae were found to be ESBL producers.

In conclusion, the overall findings of this study suggest that HiCrome UTI agar media offers an excellent media for routine urine culture. Our study compared the efficacy of HiCrome UTI agar with conventional media. The chromogenic media support the growth of all u ropathogens and differentiation of mixed culture in primary culture plate as opposed to conventional culture system.

All isolates of *Enterococcus*, E. coli, *Klebsiella*, Proteus and Acinetobacter were easily identified on HiCrome agar based on greater color differentiation. HiCrome media have an advantage over conventional media for identification of *Enterococcus* and E. coli. Since the use of chromogenic medium reduce the need for further subculture and extra confirmatory tests.

HiCrome media significantly reduce the workload and turnaround time in microbiology laboratory compared to that BA and MA agar plates. Direct biochemical reaction and ABST test can be performed from primary plate itself. HiCrome media are still expensive at the moment but considering the overall cost of multiple media and different biochemical tests necessary to identify organisms in the conventional system, it is cost effective. In conclusion HiCrome UTI agar is recommended as single medium for direct isolation, presumptive identification.

Table.1 Urine culture results among study population (N=874)

Culture Results	Number	Percentage
Bacterial growth	481	55%
No bacterial growth	393	44.9%

Table.2 Type of growth among positive isolates (N= 481)

Growth	Number	Percentage
Monomicrobial	413	85.8%
Polymicrobial	68	14.1%

Table.3 Distribution of Gram negative, gram positive and other isolates (N=481)

Organisms	Number	Percentage
Gram negative	348	72.3%
Gram positive	81	16.8%
Yeasts	52	10.8%

Table.4 Age wise distribution of patient population (N= 481)

Age wise Distribution	Number of Patients	Percentage
<20 yrs.	62	12.8%
21-40 yrs.	205	42.6%
41-60yrs	156	32.4%
>60	58	12.5%

Table.5 Gender wise distribution of patient population (N=481)

Gender	Number of Patients	Percentage
Female	322	66.9%
Male	159	33.5%

Table.6 Distribution of organisms isolated (N= 481)

Name of organisms	Number	Percentage
E.coli	150	31.1%
Klebsiella pneumonia	97	20.1%
Enterococcus faecalis	51	10.6%
Pseudomonas aeruginosa	36	7.4%
Enterobacter spp	27	5.6%
Staphylococcus aureus	21	4.3%
Acinetobacter spp	13	2.7%
Citrobacter spp	10	2.0%
Proteus spp	9	1.8%
Streptococcus agalactiae	9	1.8%
Serratia spp	6	1.2%
Yeast	52	10.8%

Table.7 Comparison of three culture media for the rate of isolation (N=481)

Isolates	Total No	HiCrome agar		Blood agar		MaConkey agar	
	N	N	%	N	%	N	%
Eschericia.coli	150	150	100%	150	100%	150	100%
Klebsiella pneumonia	97	97	100%	97	100%	97	100%
Enterococcus faecalis	51	51	100%	49	96%	26	50.9%
Pseudomonas aeruginosa	36	36	100%	36	100%	36	100%
Staphylococcus aureus	21	21	100%	21	100%	21	100%
Acinetobacter spp	13	13	100%	13	100%	13	100%
Streptococcus agalactiae	9	9	100%	9	100%	6	66.6%
Serratia spp	6	6	100%	6	100%	6	100%
Proteus spp	9	9	100%	9	100%	9	100%
Citrobacter spp	10	10	100%	10	100%	10	100%
Enterobacter spp	27	27	100%	26	96%	27	100%
Total	429	429	100%	426	99%	401	93%

Table.8 Rate of presumptive identification (N=481)

Isolate	**Total No**	**HiCrome Agar**		**BA**		**MA**	
		N	**%**	**N**	**%**	**N**	**%**
Eschericia.coli	150	150	100%	138	92%	142	94%
Klebsiella pneumonia	97	97	100%	94	97%	95	98%
Enterococcus faecalis	51	51	100%	46	90%	19	37%
Pseudomonas aeruginosa	36	36	100%	31	86.1	32	88%
Staphylococcus aureus	21	21	100%	17	81%	12	57%
Acinetobacter spp	13	13	100%	0	0%	0	0%
Streptococcus agalactiae	9	9	100%	7	77%	4	44%
Serratia spp	6	6	100%	6	100	6	100
Proteus spp	9	9	100%	9	100	9	100
Citrobacter spp	10	7	70%	0	0%	0	0%
Enterobacter spp	27	23	85%	0	0%	0	0%
Total	429	422	98%	348	81%	319	74%

Table.9a Organisms isolated from mixed growth in various media (N=34)*

Organisms	*Number*	*Blood agar*	*MacConkey agar*	*HiCrome UTI agar*
E.coli+Enterococcus	*10*	*10*	*0*	*10*
E.coli+Acinetobacter	*6*	*6*	*6*	*6*
Klebsiella+E.coli	*3*	*3*	*3*	*3*
Enterococcus+staph.aureus	*2*	*2*	*0*	*2*
E.coli+Proteus	*1*	*1*	*1*	*1*
Klebsiella+Acinetobacter	*1*	*0*	*1*	*1*
E.coli+Pseudomonas	*2*	*2*	*2*	*2*
Klebsiella+Pseudomonas	*1*	*1*	*1*	*1*
Klebsiella+Enterococcus	*3*	*3*	*0*	*3*
E.coli +S.agalactiae	*1*	*1*	*0*	*1*
Pseudomonas+Enterococcus	*2*	*2*	*0*	*2*
E.coli+Enterobacter	*1*	*0*	*1*	*1*
E.coli+staph.aureus	*1*	*1*	*0*	*1*
Total	*34*	*32*	*15*	*34*

* 34 cases yielded a total of 68 bacteria.

Table.9b Showing rate of detection of mixed growth in media (N=34)

Media	Number	Percentage %
HiCrome UTI agar	34	100%
BA	32	94%
MA	15	44.1%

Table.10a Antibiogram of Escherichia. Coli (N=150)

Antibiotics	**Sensitive**		**Resistance**		**Intermediate**	
	NO	**%**	**NO**	**%**	**NO**	**%**
Ampicillin	0	0%	150	100%	-	-
PiperacillinTazobactam	128	85%	22	14%	-	-
Aztreonam	88	58%	62	41.3%	-	-
Ceftriaxone	63	42%	84	56%	3	2%
Cefipime	81	54%	69	46%	-	-
Cefoperazonesulbactam	127	84%	22	14.6%	1	0.6%
Cefipime Tazobactam	130	86%	20	13.3%	-	-
Imipenem	132	88%	15	10%	3	2%
Meropenem	131	87%	17	11.3%	2	1.3%
Ciprofloxacin	80	53%	66	44%	4	2.6%
Levofloxacin	84	56%	52	34.6%	14	9.3%
Gentamicin	111	74%	34	22.6%	5	3.3%
Amikacin	115	76%	17	11.3%	18	12%
Doxycycline	87	58%	59	39.3%	4	2.6%
Cotrimoxazole	81	54%	69	46%	-	-
Fosfomycin	144	96%	6	4%	-	-
Nitrofurantoin	139	92%	8	5.3%	3	2%

Table.10b Multidrug resistance pattern of Escherichia. Coli (N=150)

MDR	**NO OF ISOLATES**	**PERCENTAGE**
ESBL	48	32%
CRE	18	12%

Table.11a Antibiogram of *Klebsiella* pneumonia (N=97)

Antibiotics	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
PiperacillinTazobactam	54	55%	1	1.03%	42	43.2%
Aztreonam	38	39%	-	-	59	60.8%
Ceftriaxone	34	35%	-	-	63	65%
Cefipime	38	39%	-	-	59	60.8%
Cefoperazonesulbactam	54	55%	1	1.03%	42	43.2%
Cefipime Tazobactam	55	56%	15	15%	27	27.8%
Imipenem	41	42%	15	15.4%	26	26.8%
Meropenem	56	57%	-	-	41	42.2%
ciprofloxacin	45	46.3%	1	1.03%	51	52.5%
Levofloxacin	45	46.3%	10	10.3%	42	43.2%
Gentamicin	52	53.6%	-	-	45	46.3%
Amikacin	55	56.7%	5	5.15%	37	38%
Doxycycline	46	47.4%	5	5.15%	46	47.4%
Cotrimoxazole	40	41.2%	-	-	57	58.7%
Nitrofurantoin	26	26.8%	10	10.3%	61	62.8%

Table.11b Multidrug resistant pattern of *Klebsiella* pneumonia (N=97)

MDR	NO OF ISOLATES	PERCENTAGE
ESBL	17	17.5%
CRE	42	43.2%

Note: Ampicillin was not reported for *Klebsiella* pneumoniae isolates due to intrinsic resistance.

Table.12 Antibiogram of Pseudomonas aeruginosa (N=36)

Antibiotics	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
PiperacillinTazobactam	22	61%	-	-	14	38.8%
Aztreonam	26	72%	-	-	10	27.7%
Cefipime	20	55.5%	-	-	16	44.4%
Cefoperazonesulbactam	22	61%	-	-	14	38.8%
Cefipime Tazobactam	22	61%	-	-	14	38.8%
Imipenem	22	61%	-	-	14	38.8%
Meropenem	22	61%	-	-	14	38.8%
Ciprofloxacin	21	58.3%	1	2.7%	14	38.8%
Levofloxacin	21	58.3%	1	2.7%	14	38.8%
Gentamicin	20	55.5%	2	5.5%	14	38.8%
Amikacin	22	61%	-	-	14	38.8%
Nitrofurantoin	-	-	-	-	36	100%
Ceftazidime	26	72.2%	-	-	10	27.7%

Table.13 Antibiogram of Enterobacter (n=27)

Antibiotics	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
Ampicillin	-	-	0	0%	27	100%
PiperacillinTazobactam	24	88.8%	1	3.7%	2	7.4%
Aztreonam	23	85%	-	-	4	14.8%
Ceftriaxone	21	77%	-	-	6	22.2%
Cefipime	23	85%	-	-	4	14.8
Cefoperazonesulbactam	24	88.8%	-	-	3	11.1%
Cefipime Tazobactam	25	92.5%	1	3.7%	1	3.7%
Imipenem	25	92.5%	1	3.7%	1	3.7%
Meropenem	25	92.5%	1	3.7%	1	3.7%
ciprofloxacin	23	85%	2	7.4%	2	7.4%
Levofloxacin	26	96.2%	-	-	1	3.7%
Gentamicin	23	85%	1	3.7%	3	11.1%
Amikacin	24	88.8%	-	-	3	11.1%
Cotrimoxazole	23	85%	-	-	4	14.8%
Minocycline	25	92.5%	-	-	2	7.4%
Nitrofurantoin	15	55.5%	-	-	12	44.4%

Note: Ampicillin was not reported for Enterobacter due to intrinsic Resistance.

Table.14 Antibiogram of Acinetobacter (N=13)

Antibiotics	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
PiperacillinTazobactam	12	92%	1	7.6%	-	-
Ceftriaxone	2	15%	5	38%	6	46.1%
Cefipime	9	69%	-	-	4	30.7%
Cefoperazonesulbactam	13	100%	-	-	-	-
Cefipime Tazobactam	13	100%	-	-	--	-
Imipenem	11	84%	-	-	2	15%
Meropenem	11	84%	-	-	2	15%
Ciprofloxacin	12	92%	-	-	1	7.6%
Levofloxacin	12	92%	-	-	1	7.6%
Gentamicin	11	84%	-	-	2	15%
Amikacin	11	84%	1	7.6%	1	7.6%
Doxicycline	9	69%	-	-	4	30.7%
Nitrofurantoin	1	7.6%	-	-	12	92.3%

Note: Ampicillin, Fosfomycin, Aztreonam was not reported for Acinetobacter due to intrinsic Resistance.

Table.15 Antibiogram of Citrobacter (N=10)

Antibiotics	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
Ampicillin	-	-	-	-	10	100%
PiperacillinTazobactam	10	100%	-	-	-	-
Aztreonam	10	100%	-	-	-	-
Ceftriaxone	9	90%	-	-	1	10%
Cefipime	10	100%	-	-	-	-
Cefoperazonesulbactam	10	100%	-	-	-	-
Cefipime Tazobactam	10	100%	-	-	-	-
Imipenem	10	100%	-	-	-	--
Meropenem	10	100%	-	-	-	-
ciprofloxacin	10	100%	-	-	-	-
Levofloxacin	10	100%	-	-	-	-
Gentamicin	10	100%	-	-	-	-
Amikacin	10	100%	-	-	-	-
Doxicycline	9	90%	-	-	-	-
Cotrimoxazole	9	100%	-	-	1	10%
Nitrofurantoin	8	80%	1	10%	1	10%

Table.16 Antibiogram of Proteus (N=9)

Antibiotics	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
PiperacillinTazobactam	9	100%	-	-	-	-
Aztreonam	8	88%	-	-	1	11%
Ceftriaxone	8	88%	-	-	1	11%
Cefipime	8	88%	-	-	1	11%
Cefoperazonesulbactam	9	100%	-	-	-	-
Cefipime Tazobactam	9	100%	-	-	-	-
Imipenem	9	100%	-	-	-	-
Meropenem	9	100%	-	-	-	--
Ciprofloxacin	8	88%	-	-	1	11%
Levofloxacin	8	88%	-	-	1	11%
Gentamicin	8	88%	-	-	1	11%
Amikacin	9	100%	-	-	-	-
Cotrimoxazole	5	55%	-	-	4	44%
Nitrofurantion	-	-	-	-	9	100%

Table.17a Antibiogram of Staphylococcus aureus (n=21)

Antibiotics	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
Penicillin	14	66%	-	-	7	33%
Cefoxitin	14	66%	-	-	7	33%
Cotrimoxzsole	19	90%	-	-	2	9.5%
Ciprofloxacin	10	47%	-	-	11	52%
Moxifloxacin	19	90%	2	9.5%	-	-
Tetracyclin	-	--	-	-	3	14%
Linezolid	21	100%	-	-	-	-
Rifampicin	20	95%	-	-	1	4.7%
Minocycline	21	100%	-	-	-	-
Nitrofurantoin	21	100%	-	-	-	-

Table.17b Multidrug resistance pattern of Staphylococcus aureus (N=21)

RESISTANCE	NUMBER OF ISOLATES	PERCENTAGE
MRSA	7	33.3%
MSSA	14	66.6%%

Table.18 Antibiogram of *Enterococcus* spp (N=51)

Antibiotics	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
Vancomycin	51	100%	-	-	-	-
Linezolid	51	100%	-	-	-	-
Teicoplanin	50	98%	-	-	1	1.9%
Levofloxacin	26	50.9%	1	1.9%	24	47%
Tetracyclin	12	23%	-	-	39	76%
Fosfomycin	37	72%	2	3.9%	12	23%
HLG	25	49%	-	-	26	50.9%
Ampicillin	32	62.7%	-	-	19	37%
Nitrofurantoin	37	72.5%	-	-	14	27%

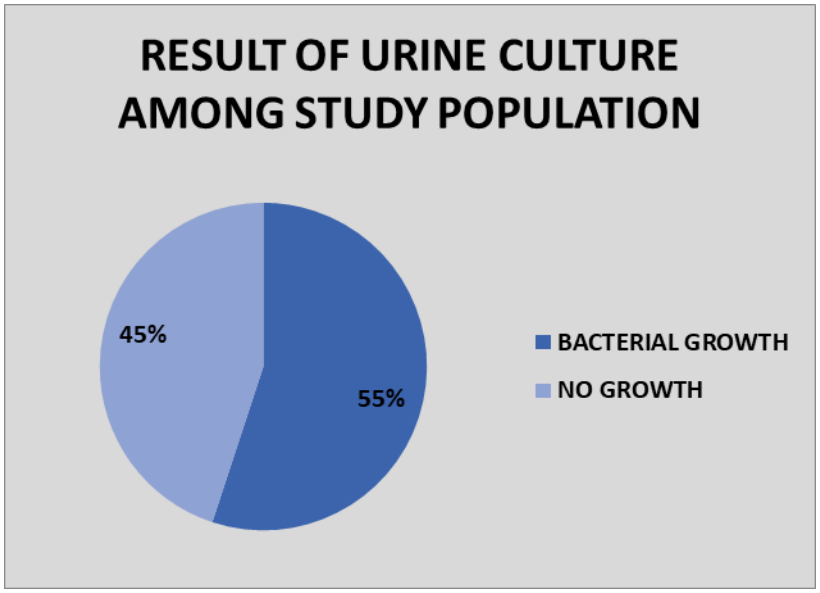
Table.19 Antibiogram of Streptococcus agalactiae (N=9)

Antibiotics	Sensitive		Intermediate		Resistant	
	NO	%	NO	%	NO	%
Vancomycin	9	100%	-	-	-	-
linezolid	9	100%	-	-	-	-
Teicoplanin	9	100%	-	-	-	-
Levofloxacin	9	100%	-	-	-	-
Tetracyclin	9	100%	-	-	-	--
Ampicillin	8	88.8%	-	-	1	11.1%
Nitrofurantoin	9	100%	-	-	-	-
Cotrimoxazole	-	-	-	-	9	100%
Ceftriaxone	9	100%	-	--	-	-

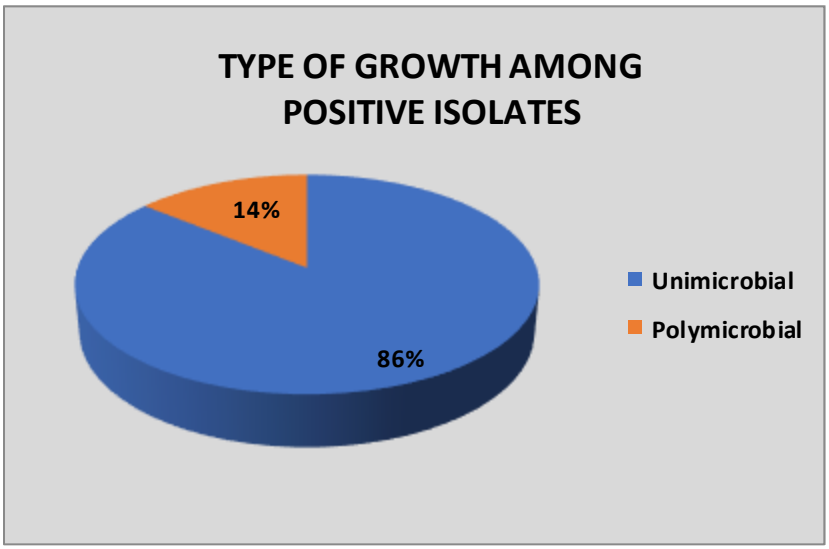
Table.20 Distribution of MDR isolates

CRE	**77**	**51.3%**
ESBL	66	44%
MRSA	7	4.6%

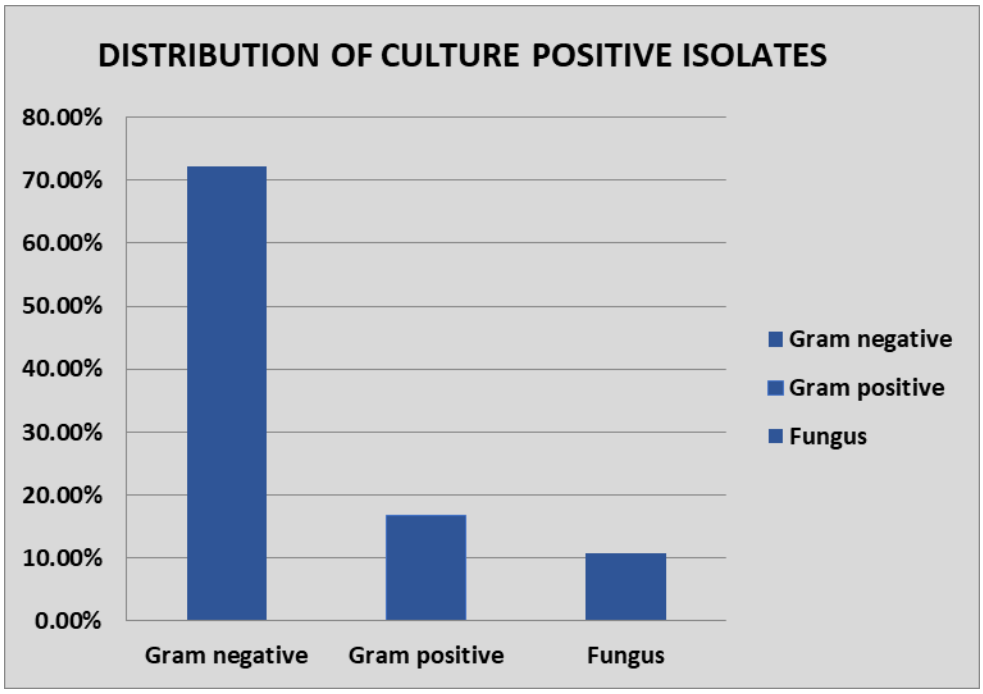
Graph.1 Result of urine culture among study population



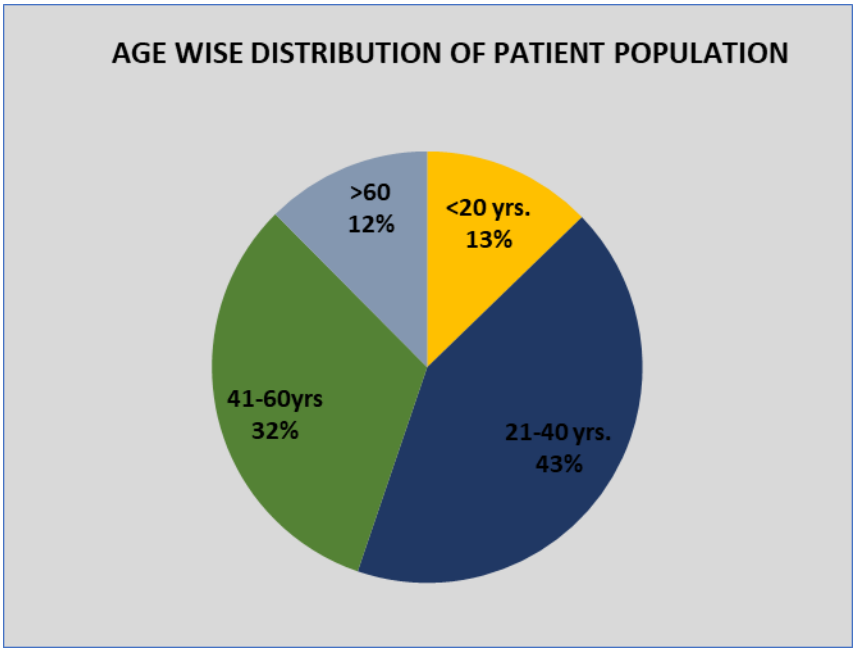
Graph.2 Type of growth among positive isolates



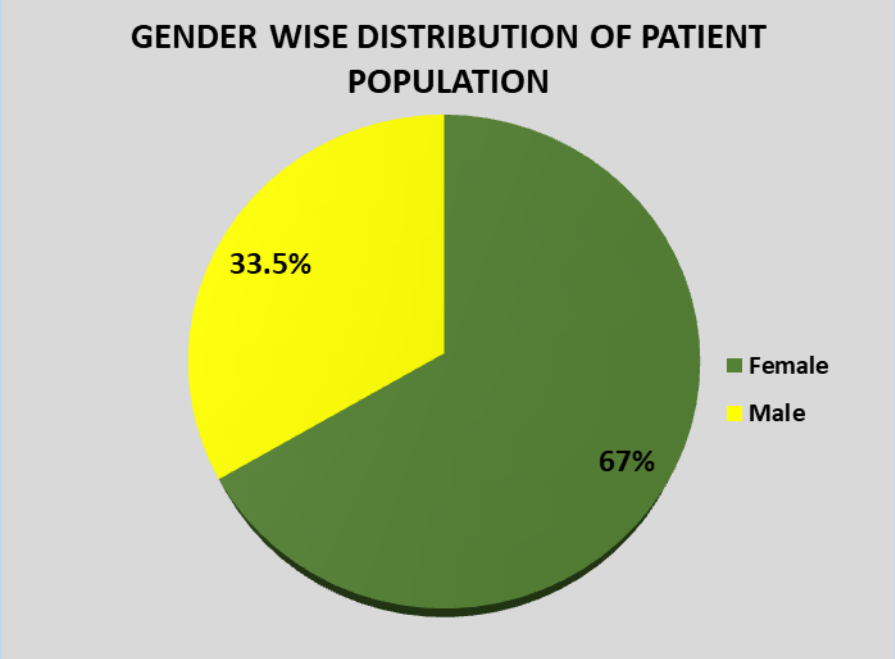
Graph.3 Distribution of culture positive isolates



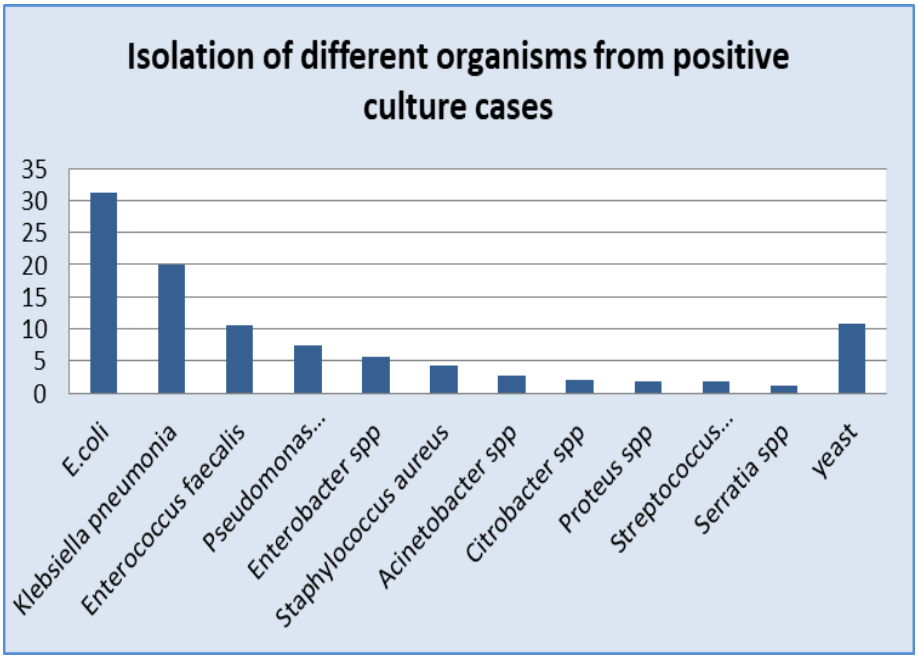
Graph.4 Age wise distribution of patient population



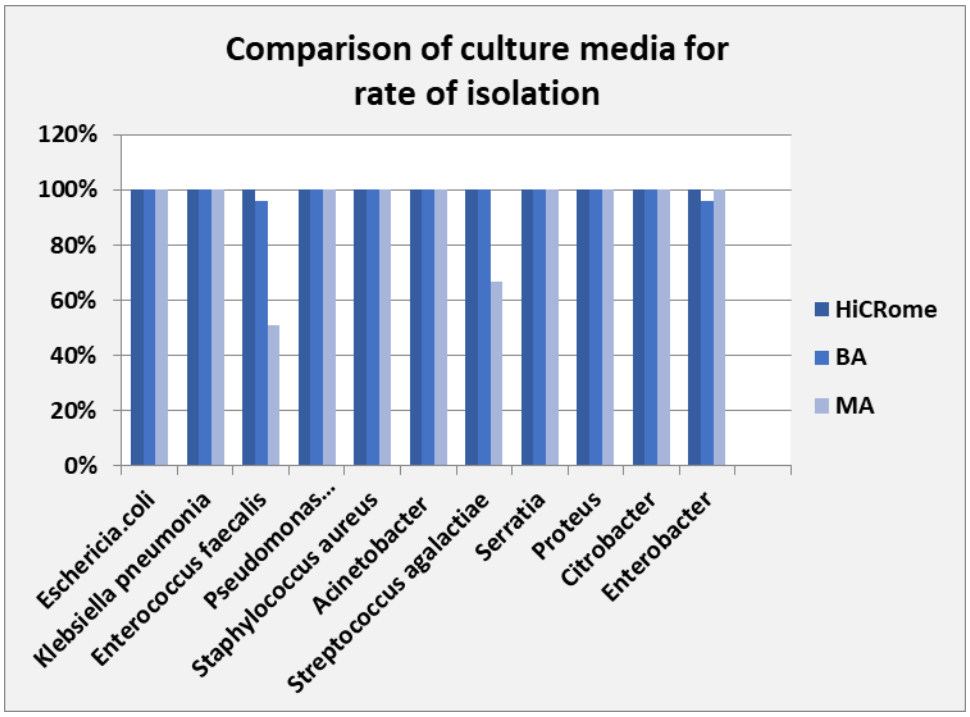
Graph.5 Gender wise distribution of patient population



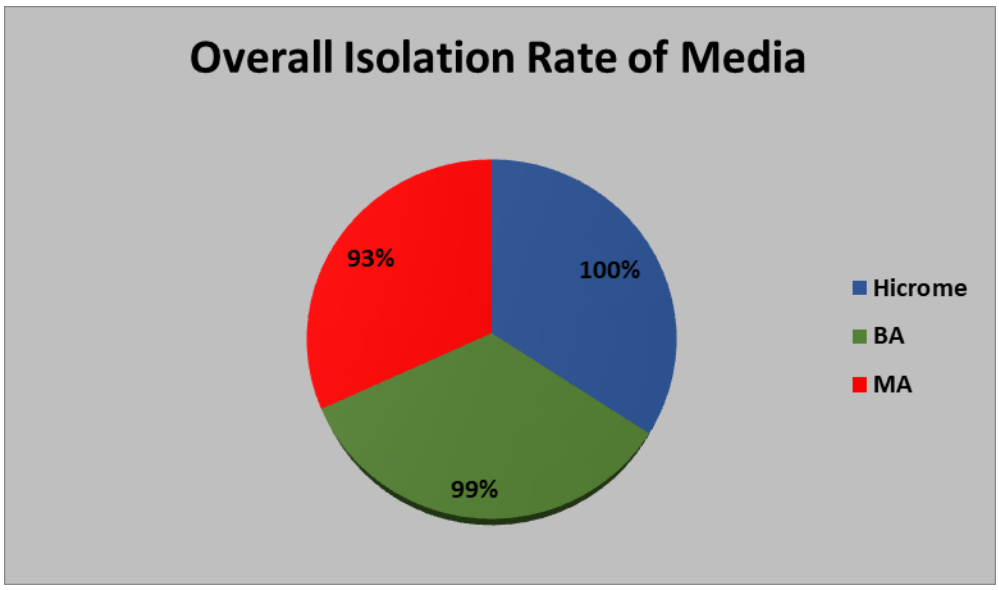
Graph.6 Distribution of organisms isolated



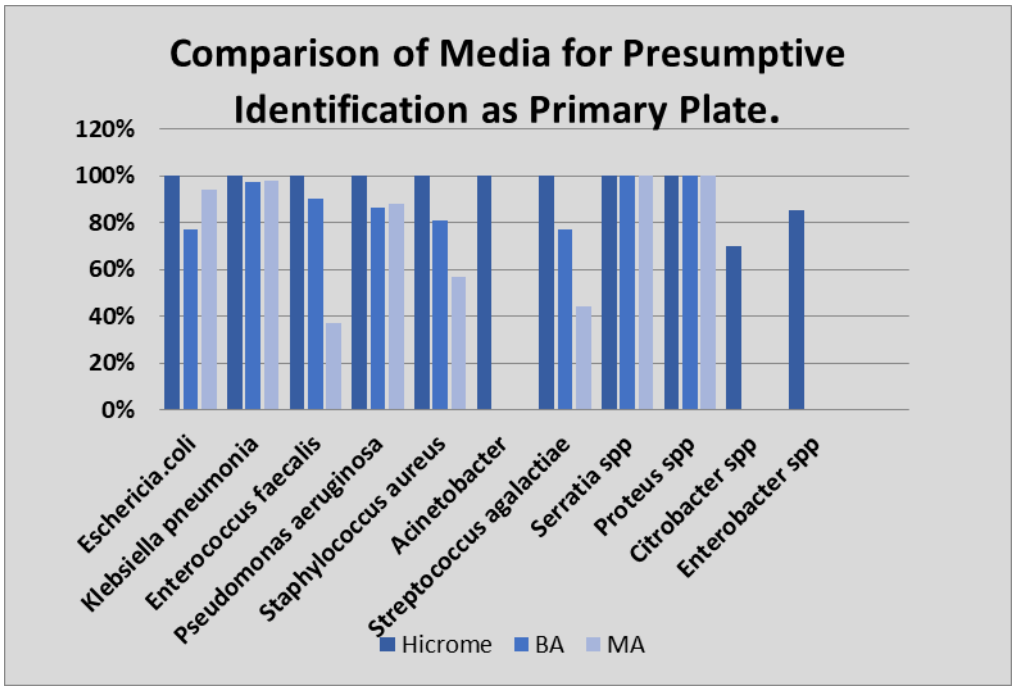
Graph.7a Comparison of culture media for rate of isolation



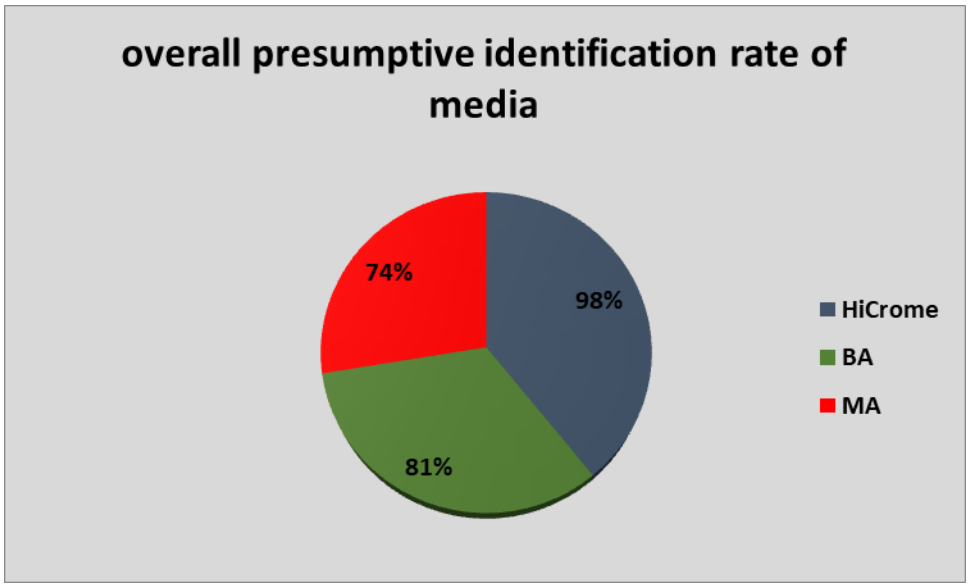
Graph.7b Overall isolation rate of media



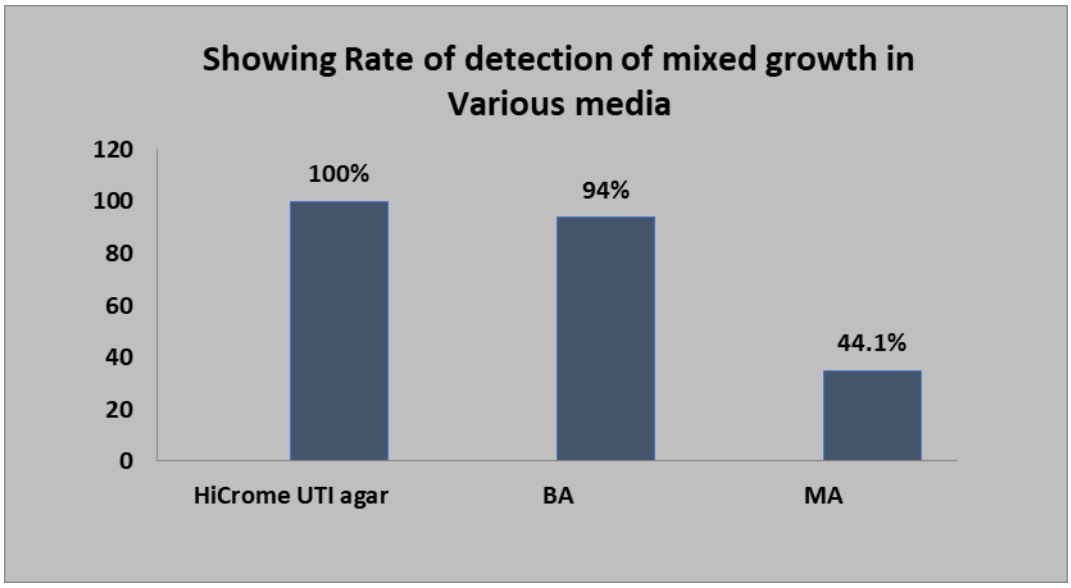
Graph.8a Comparison of media for presumptive identification as primary plate



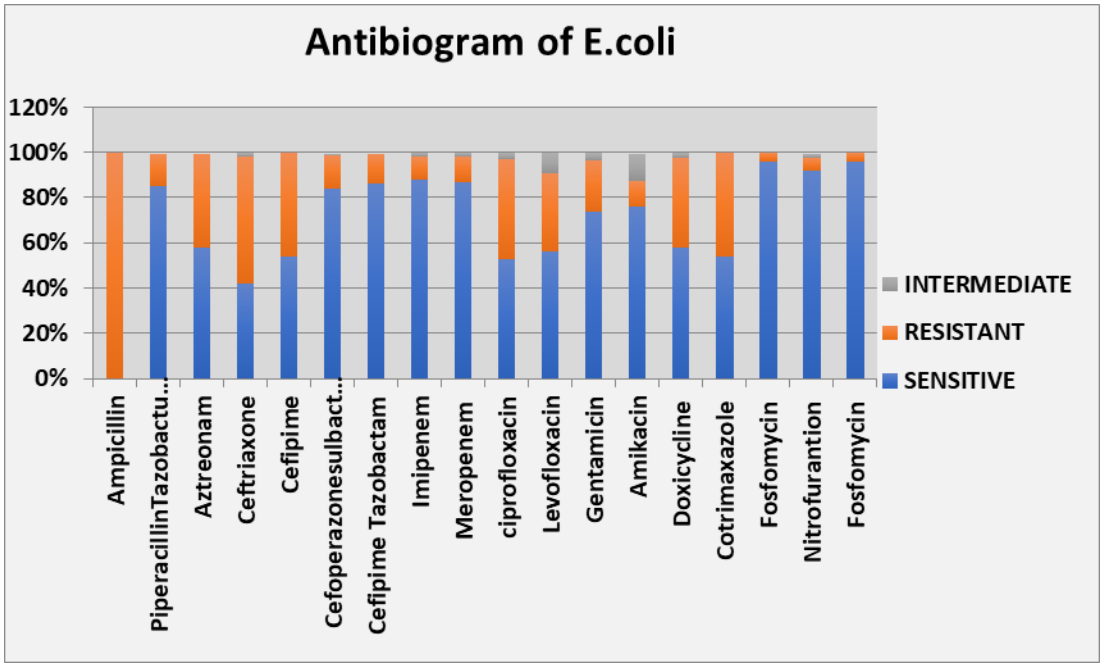
Graph.8b Overall presumptive identification rate of media



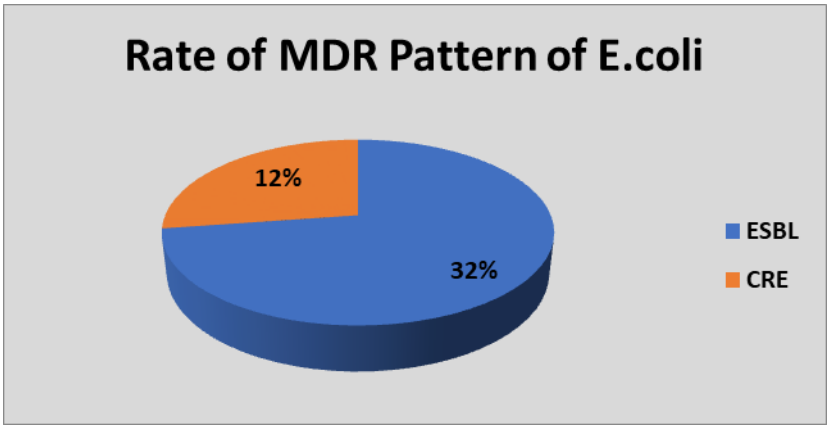
Graph.9 Rate of detection of mixed growth in various media



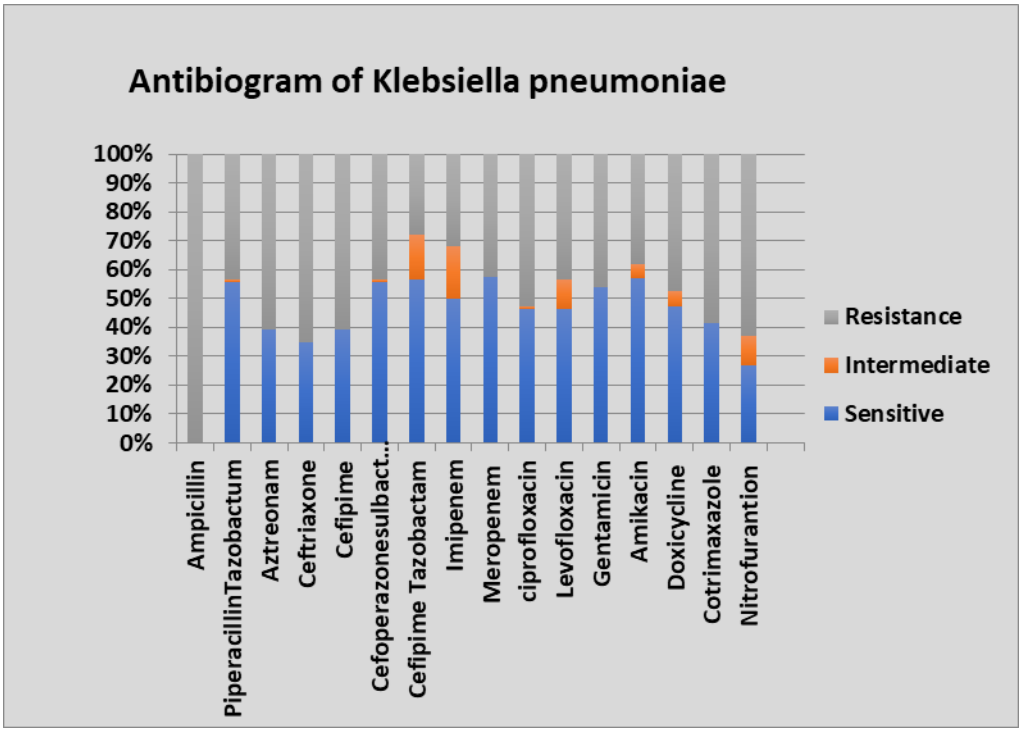
Graph.10a Antibiogram of E.coli.



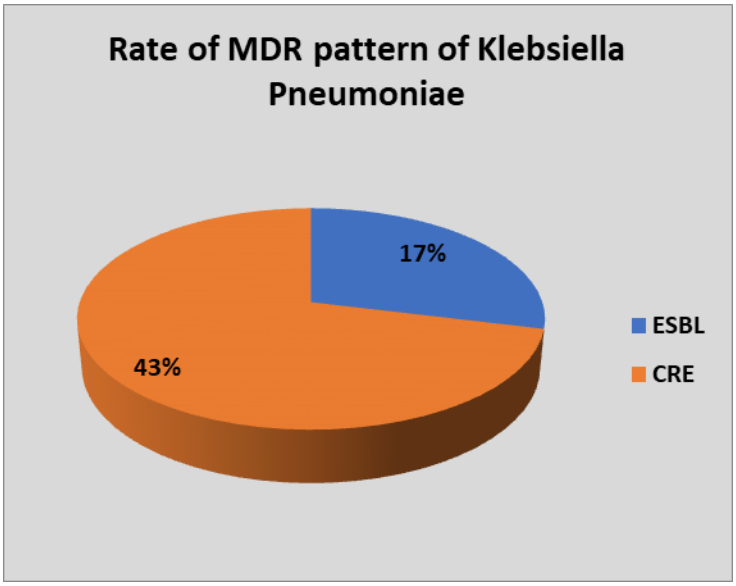
Graph.10b Rate of MDR pattern of E.coli



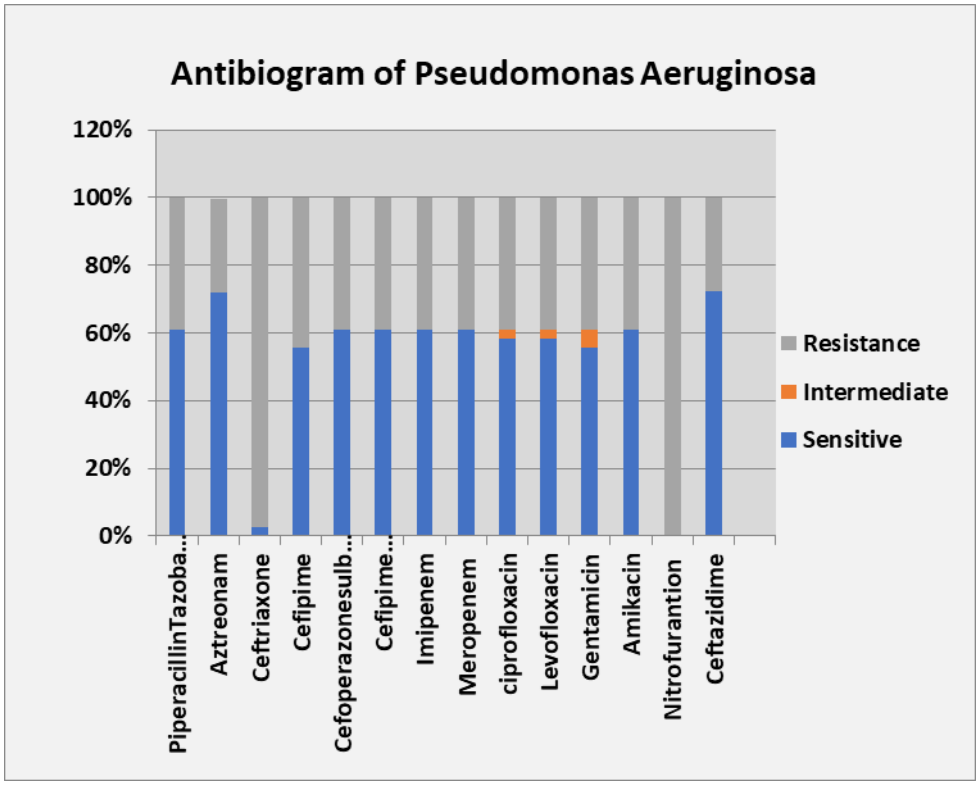
Graph.11a Antibiogram od *Klebsiella* pneumoniae



Graph.11b Rate of MDR pattern of *Klebsiella* pneumoniae

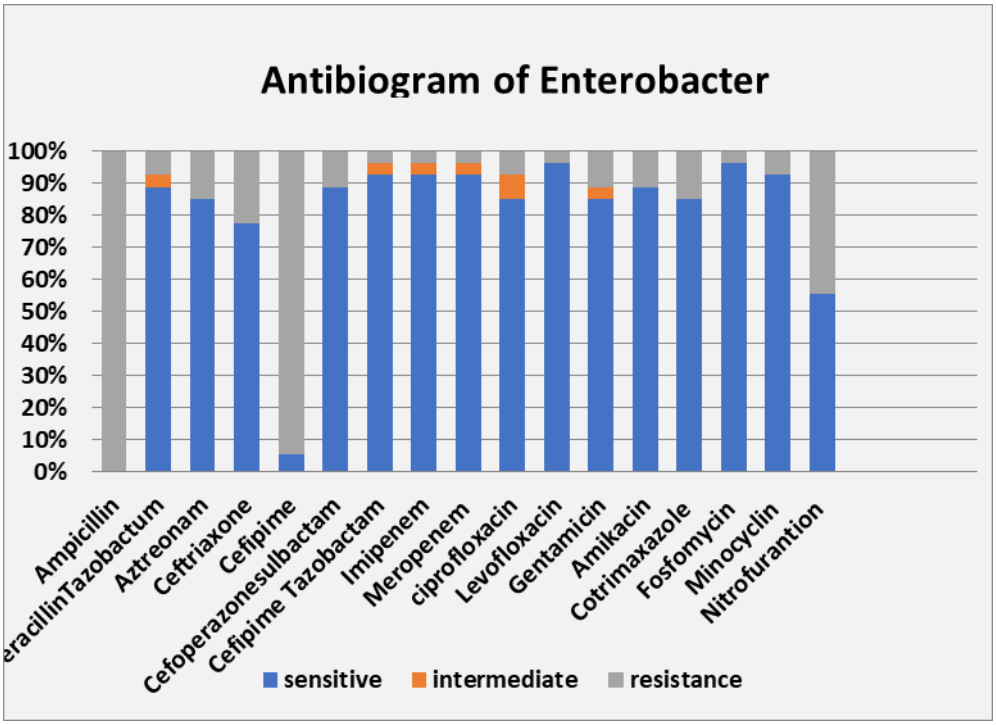


Graph.12 Antibiogram of Pseudomonas aeruginos

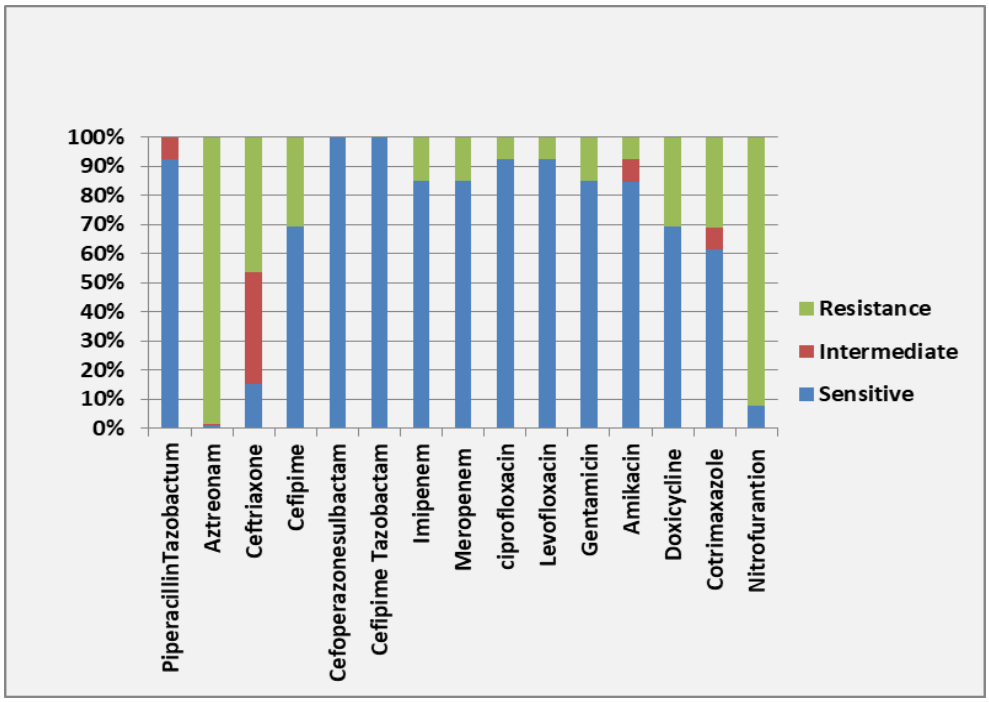


Note: Tigecyclin, Doxycycline, Cotrimoxazole, Ampicillin, Minocycline was not reported for Pseudomonas spp due to intrinsic resistance.

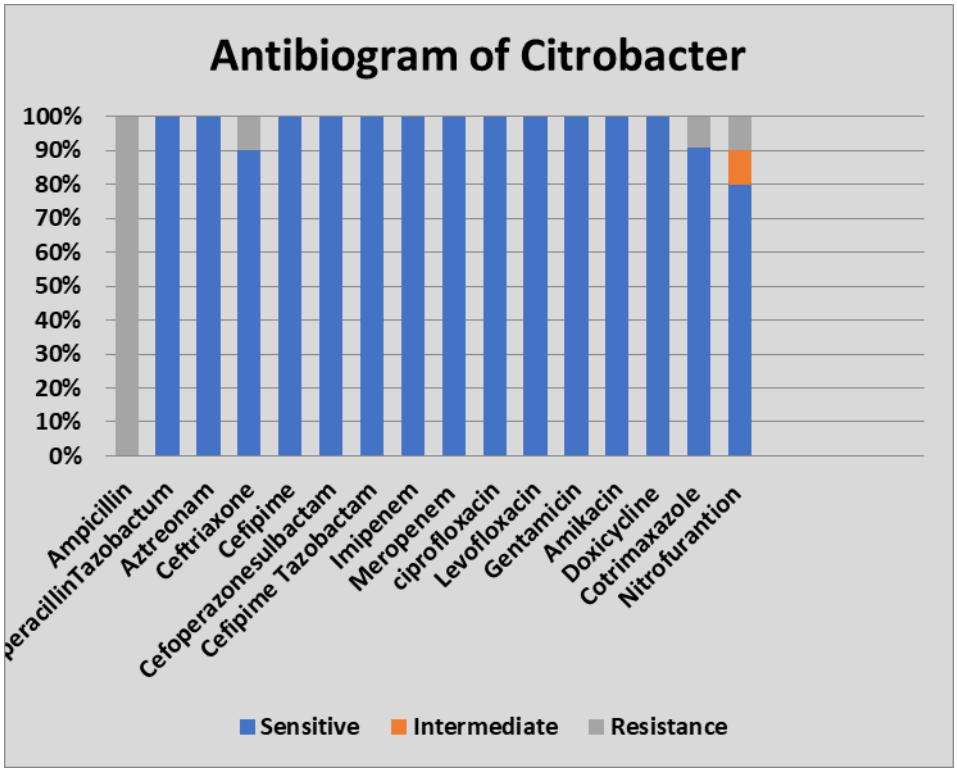
Graph.13 Antibiogram of Enterobacter



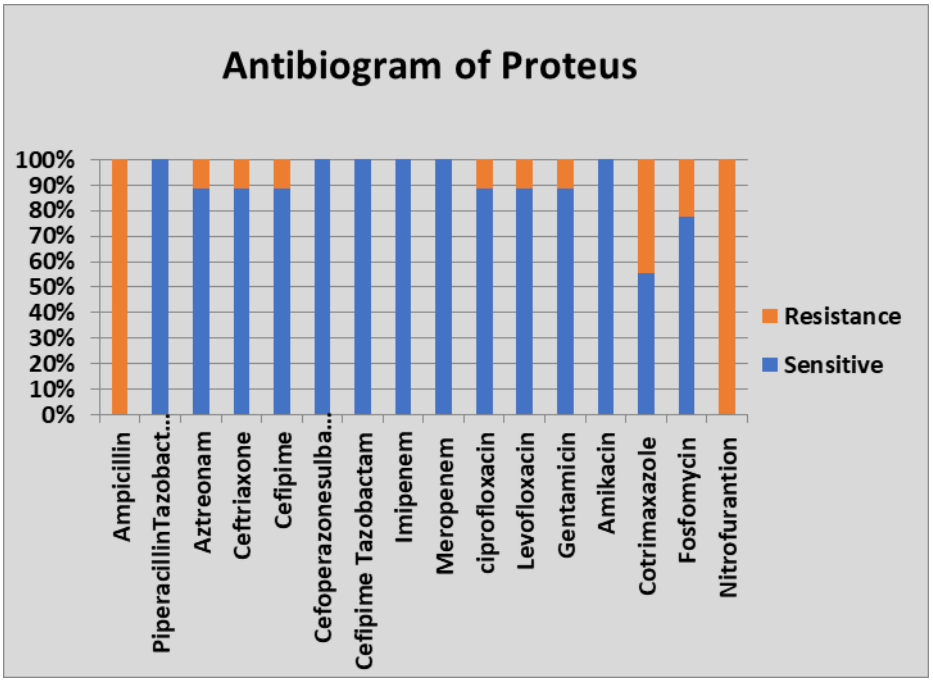
Graph.14 Antibiogram of Acinetobacter



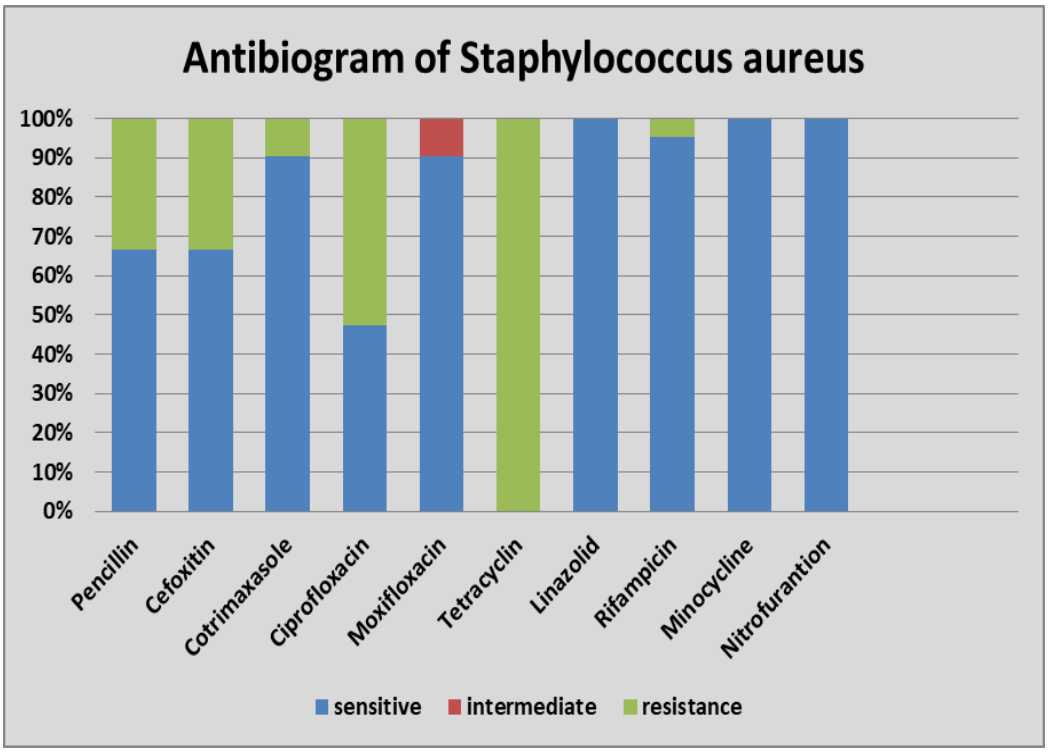
Graph.15 Antibiogram of Citrobacter



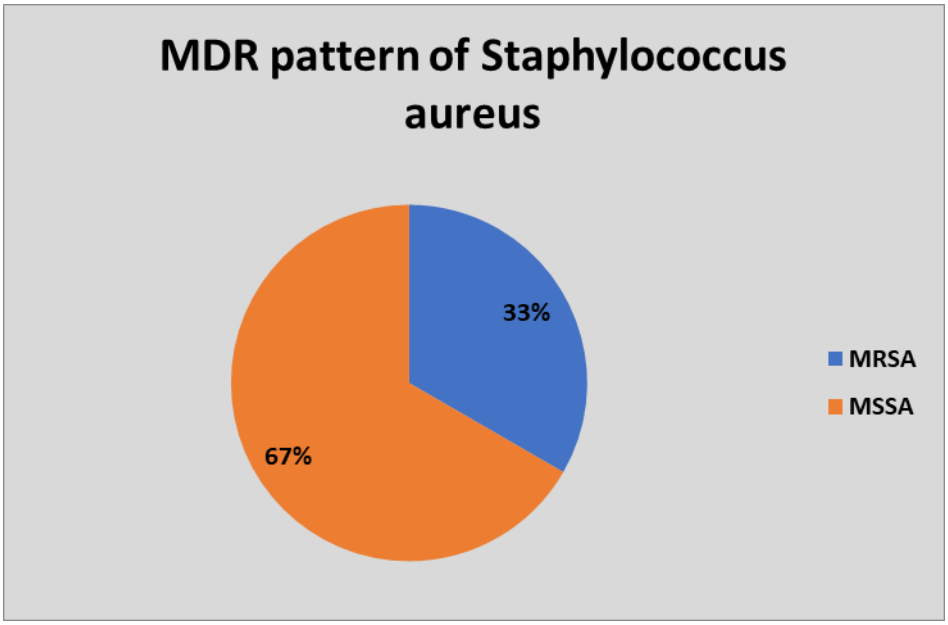
Graph.16 Antibiogram of Proteus



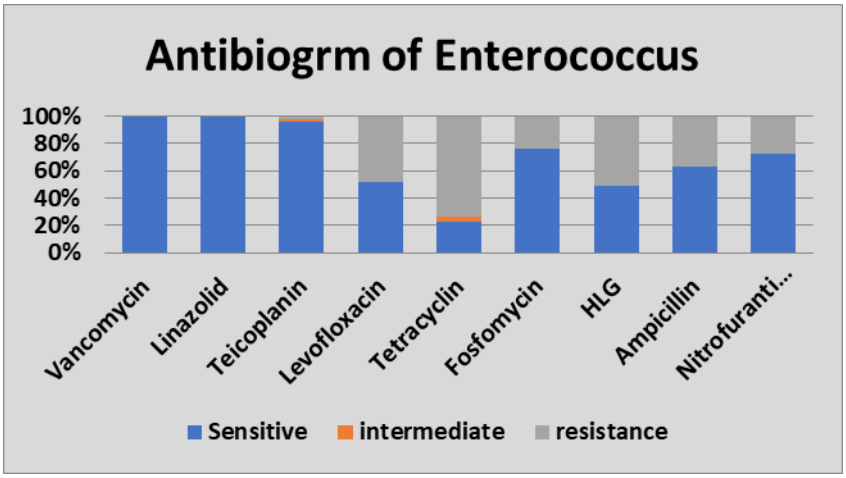
Graph.17a Antibiogram of Staphylococcus aureus



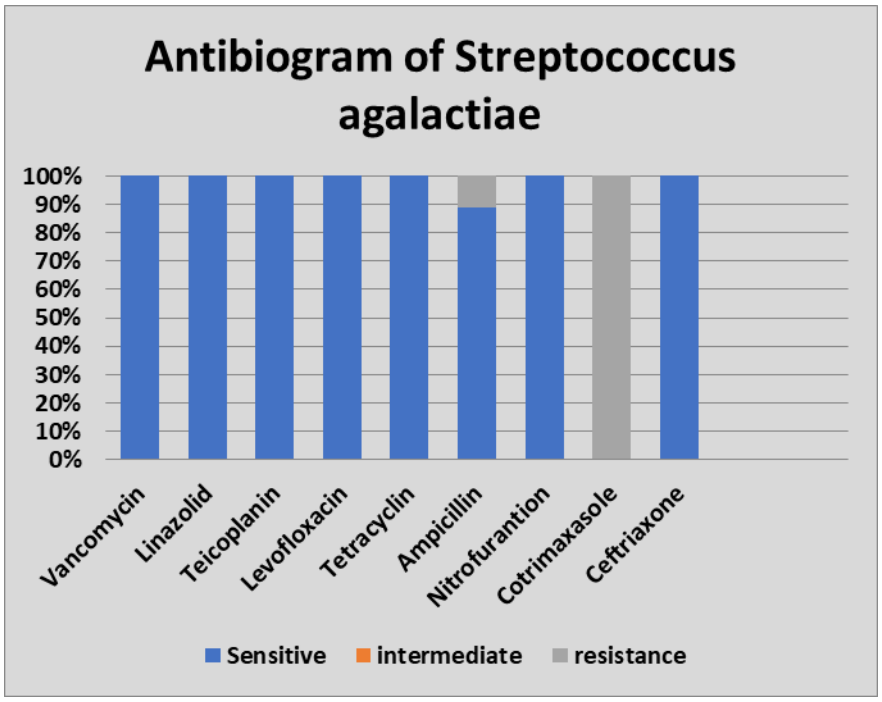
Graph.17b MDR Pattern of Staphylococcus aureus



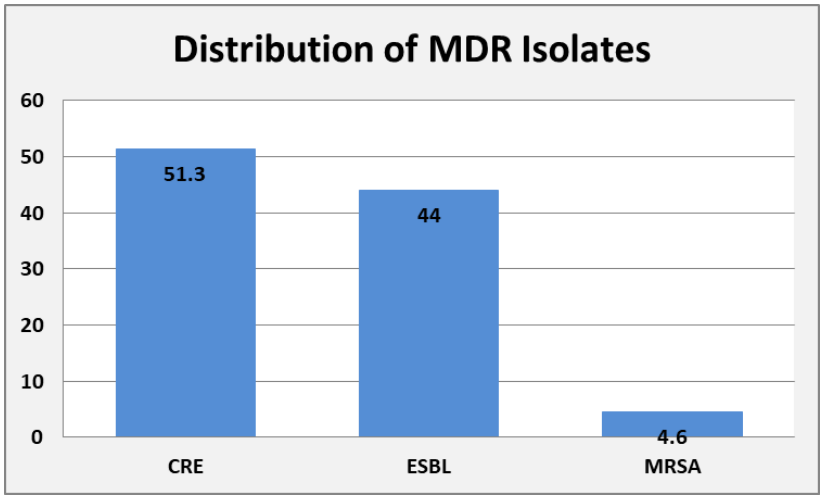
Graph.18 Antibiogram of *Enterococcus* spp



Graph.19 Antibiogram of Streptococcus agalactiae



Graph.20 Distribution of MDR isolates



The Higher prevalence of urinary tract infection was seen in females than males. E. coli was the predominant pathogen responsible for Urinary tract infection followed by *Klebsiella* pneumoniae. Abuse of antibiotics and empirical management of UTIs causing some drugs to become less sensitive to certain microorganisms than was previously reported. Antibiotic resistance of uropathogens for most of the important antibiotics was very high. The present study observed the high prevalence of multidrug resistance among bacterial uropathogens. Particularly, rate of resistance to carbapenems and third generation cephalosporins were higher. Higher percentage of ESBL producing E. coli and carbapenem resistant *Klebsiella* pneumonia was seen in this study. Early detection and close monitoring of MDR must be started by all clinical microbiology laboratories to reduce the antimicrobial resistance that is now a global problem.

Acknowledgement

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Author Contributions

Sifana Thasni K: Conceived the original idea and designed the model and Analysed, Investigated and wrote the manuscript. Prof. Prabhisha KP: Validation, formal Analysis, project Administration, supervision. Dr. Reshmi Goplalakrishnan: Manuscript review and editing. Prof. Savitha M: Manuscript formatting and citation management.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding

author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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