

## A clinical study to examine the resistance pattern of culture isolates to Amoxicillin Clavulanic acid (AMC) in the sample sent to culture sensitivity: A teaching Hospital based study at north India

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### Abstract

**Background:** Concurrent administration of a  $\beta$ -lactamase inhibitor such as clavulanate or sulbactam markedly expands the spectrum of activity of acid resistant penicillins like ticarcillin and piperacillin. The dose as well as the incidence of toxicity were subsequently reduced with semi-synthetic penicillins like ticarcillin, which makes it the preferred ureidopenicillin against *P. aeruginosa* infections. Amoxicillin Clavulanic acid (AMC) is a broad-spectrum antibacterial being used over 20 years. Emergence of resistant bacteria has complicated the empirical therapy of infections. Resistance to Amoxicillin is a matter of concern. Clavulanic acid, a  $\beta$ -lactamase inhibitor, is used to overcome resistance. But appearance of resistance to even this combination is a matter of even more concern. Hence an attempt is made to find out the proportion of resistance to AMC in a tertiary care setup. **Aim:** To examine the resistance pattern to Amoxicillin Clavulanic acid (AMC) among organisms isolated from pus sample collected for culture sensitivity. **Material and Methods:** This is the descriptive study, taken up to evaluate the resistance pattern of culture isolates of pus samples from various departments. Pus culture and sensitivity reports obtained by Kirby Bauer Disc diffusion method of pus samples during a period of 6 months was collected from Microbiology Department. **Results:** A total of 250 reports were analyzed. 250 reports included in the study, 76 (30%) were reported sensitive to AMC whereas 174 (70%) were reported resistant. Of all the isolates, *Citrobacter* showed the highest rate of resistance to AMC (100%), followed by *E. coli* (87.75%), *Klebsiella* (79.31%), *Proteus* (75%), *Pseudomonas* (63.88%), *Staphylococci* (62.6%) and *Enterobacter* (50%). All the *Streptococci* that were isolated were sensitive to AMC. However, statistically significant resistance was identified highest in *E. coli* (87.75%,  $p=0.002$ ), followed by *Klebsiella* (79.31%,  $p=0.047$ ) and *Staphylococci* (62.6%,  $p=0.001$ ) among the isolated organisms (Table 3). None of the streptococci and acetobacter species that were isolated was found to be resistant to AMC. All of the *Citrobacter* species isolated showed resistance to AMC however it was not statistically significant ( $p=0.057$ ). *Staphylococci* which was the most commonly isolated organism had a resistance of 62.6% ( $p=0.001$ ), of sample culture isolates were found to be resistant to AMC. **Conclusion:** Our study concludes an encountered organism in pus samples to be *Staphylococcus*, followed by *E. coli*, *Pseudomonas* and *Klebsiella*. Nearly 70% of organisms causing purulent infections were resistant to AMC. Of the isolates, statistically significant resistance was shown by *E. coli* followed by *Klebsiella* and *Staphylococcus*.

**Keywords:** Antibiotic resistance, Isolated organism, Antibiotic, Amoxicillin Clavulanic acid (AMC), resistance, culture sensitivity.

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### Introduction

Multiple antibiotic resistances in bacterial populations are a pervasive and growing clinical problem, which is recognized as a threat to public health. Antibiotic susceptibility was confirmed by disk diffusion technique on Muller-Hinton medium (Becton Dickinson Microbiological Systems, Cockysville, MD), performed according to the Clinical Laboratory Standard Institute (CLSI) guidelines.[1] Unfortunately, *P. aeruginosa* demonstrates resistance to multiple antibiotics, thereby jeopardizing the selection of appropriate treatment.[2] Bacterial infections are one of the growing concerns globally, especially so when they are resistant to commonly used multiple antibiotics. Pyogenic infections can either be mono or poly microbial, aerobic and anaerobic or a mixture of both involving an average of 5-6 organisms on culture. [3] Antibiotics are undoubtedly a blessing to human civilization that has saved millions of lives fighting against microbes or infections. [4] The first  $\beta$ -

lactams antibiotics were introduced for use in the 1950s (Penicillin G and V), but they had substantial inconveniences, like a limited range of activity, a short half-life, and the administration route had to be parenteral. [5] To overcome these, amoxicillin was developed and marketed in 1972, which was a moderate spectrum  $\beta$ -lactam active against wide range of Gram positive and a limited range of Gram-negative organisms. It still remains the most commonly utilized drug in the class due to its better oral availability.[6] During the past decades many bacteria developed the ability to resist  $\beta$ -Lactam antibiotics, with  $\beta$ -Lactamase production being the most common mechanism of resistance.  $\beta$ -Lactamase produced by several Gram-positive organisms (including *Staphylococci* spp.), Gram-negative organisms (*Escherichia coli*, *Neisseria gonorrhoeae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Pseudomonas* and *Klebsiella* spp.), and anaerobic organisms (*Bacteroides* spp.) can cleave the  $\beta$ -lactam ring and render the antibiotic inactive.[7] To overcome the problem of  $\beta$ -Lactamase production, research was directed towards identifying compounds which can inhibit  $\beta$ -lactamase. In 1972, a compound called Clavulanic Acid was identified which was structurally related to penicillins and prevented inactivation of  $\beta$ -Lactam antibiotics when given together.[8] The combination of amoxicillin and

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clavulanic acid (AMC) was made available to market in 1981, which was the only penicillin and  $\beta$ -lactamase inhibitor available in oral formulation.[9] But the battle between the microbes and the antibiotics did not end there. Organisms developed mechanisms to overcome the use of  $\beta$ -Lactamase inhibitors too. The practice of antibiotic misuse and prophylactic use of antibiotics in humans as well as agricultural industries has increased exposure of antibiotics to microbes facilitating even rapid development of resistance.[10] Antimicrobial resistance in pathogens causing important communicable diseases has become a matter of great public health concern globally. The problem of resistance has emerged especially more so in developing countries like India where there is widespread availability of practically all the antimicrobials over the counter.[11] Resistance to Amoxicillin is itself a matter of concern. Clavulanic acid, a  $\beta$ -lactamase inhibitor, is used to overcome resistance. But appearance of resistance to even this combination of drugs is a matter of even more concern. The type of organism causing the infection and their sensitivity and resistance pattern vary from place to place. Therefore, appropriate selection of an effective antibiotic requires knowledge of the potential microbes and its resistance and sensitivity patterns.[12] Rapid detection and reporting of resistant organism, monitoring and providing feedback regarding antibiotic resistance and other measures are the important strategies to control antibiotic resistance in hospitals.[13] Hence this study was taken up to evaluate the pattern of resistance to AMC among organisms isolated from pus samples sent for culture sensitivity in a tertiary care hospital.

#### Material and Methods

This is a record based descriptive study, taken up in the department of Pharmacology, to evaluate the resistance pattern of culture isolates of pus samples from various departments sent to the department of microbiology.

#### Inclusion criteria

All aerobic culture and sensitivity reports obtained by Kirby Bauer Disc Diffusion method of pus samples sent to department of microbiology during the period of 6 months from Jan to June 2018 were included in the study.

#### Exclusion criteria

All samples with no isolates or those reported as contaminated were excluded from the study.

#### Methodology

After obtaining clearance from the Institutional Ethics committee, all the reports for aerobic pus culture and sensitivity obtained by Kirby Bauer Disc diffusion method from various departments were collected over a period of 6 months starting from Jan to June 2018, from the central laboratory at our institution. All the reports that did not have any isolates and all the reports reported as contaminated were excluded from the study (Table 1).

#### Statistical analysis

The data obtained were tabulated on an excel sheet and analysed using descriptive statistical methods.

**Table 1: Pattern of data obtaining.**

Total No of cases reported	Wastages(Having contaminations)	No growth	Final cases reported in the study(n=250)
450	20	180	250

#### Results

A total of 450 reports were obtained over a period of six months from Jan to June 2018. Of the 450 reports only 250 samples yielded isolates and satisfied all inclusion criteria. The data obtained from these 250 reports were tabulated and analysed using descriptive

statistical methods (n = 250). 57 isolates were present in the month of May followed by 51 isolates in Jan and 43 in the month of March. Least number of isolates was seen in the month of April accounting for 27 isolates. The month wise number of reports obtained is shown in (Table 2.)

**Table 2: Monthly Isolates**

Months	Total	Isolates available
January	74	51
February	61	32
March	68	43
April	65	27
May	97	57
June	85	40
Total	450	250

**Table 3: Percentage resistance of different organisms isolated**

Organism	Isolates Resistant	Total Isolates	Resistance (%)	p-Value
Pseudomonas	23	36	63.88	0.367
Staphylococci	72	115	62.60	0.001
Enterobacter	1	2	50	0.867
Streptococci	0	5	0	0.006
Acetobacter	0	1	0	0.673
Citrobacter	9	9	100	0.057
E.coli	43	49	87.75	0.002
Klebsiella	23	29	79.31	0.047
Proteus	3	4	75	0.763

The organism that was most commonly isolated was staphylococcus 115 followed by E. coli 49, Pseudomonas 36 and Klebsiella 29. The other organisms that were isolated were Citrobacter 9, Proteus

4, Enterobacter 2, Acetobacter 1, and Streptococci 5, (Figure 1) and (Table 3).

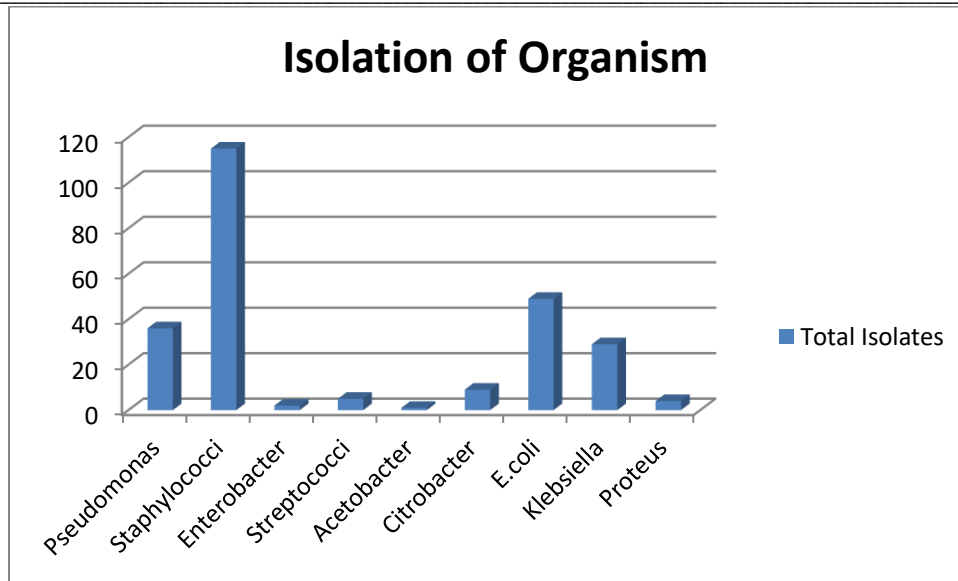


Fig 1: Organism Isolation

Of the 250 reports included in the study, 76 (30%) were reported sensitive to AMC whereas 174 (70%) were reported resistant (Figure 2).

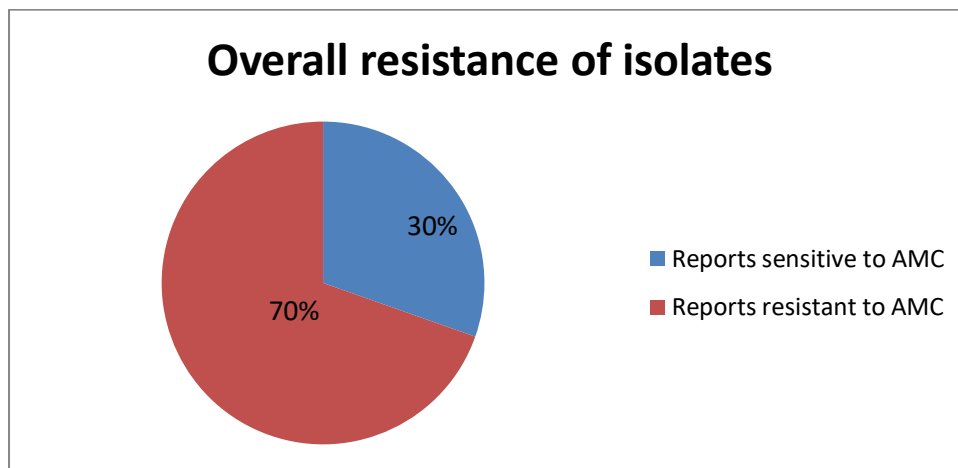


Fig 2: Resistance of isolates

Of all the isolates, Citrobacter showed the highest rate of resistance to AMC (100%), followed by E.coli (87.75%), Klebsiella (79.31%), Proteus (75%), Pseudomonas (63.88%), Staphylococci (62.6%) and Enterobacter (50%). All the Streptococci that were isolated were sensitive to AMC. However, statistically significant resistance was identified highest in E. coli (87.75%,  $p=0.002$ ), followed by Klebsiella (79.31%,  $p=0.047$ ) and Staphylococci (62.6%,  $p=0.001$ ) among the isolated organisms (Table 3). None of the streptococci and acetobacter species that were isolated was found to be resistant to AMC. All of the citrobacter species isolated showed resistance to AMC however it was not statistically significant ( $p=0.057$ ). Staphylococci which was the most commonly isolated organism had a resistance of 62.6% ( $p=0.001$ ).

#### Discussion

Identification of all isolates was carried out by a positive reaction to oxidase and production of pyocyanin. Culture examination was carried out using Nutrient agar and Mac Conkey's medium, followed by inoculation by four flame streak methods. The results of our study

correlate to findings of different authors from across the country. In a study by Bansal V *et al*, at Post Graduate Institute of Medical Education and Research, Chandigarh, India, a significant increase was noticed in the prescription of antimicrobials in 2005 as compared with 1995 (570 vs 482,  $P<0.001$ ). They noticed an increase in the use of Amoxicillin-Clavulanic acid, Metronidazole, Amikacin, and third and fourth-generation cephalosporins. Penicillin and cephalosporin remained on top of the list. They found that the combination is routinely indicated for empirical therapy of serious infections in both immune-compromised and immune competent individuals and has also found place in WHO Essential Medicine List (EML).[14] This indicates that there is an increasing trend in prescribing higher antibiotics compared to earlier days. The findings of our study correlates with the findings of the study conducted by Banu A *et al*, where 100 pus samples were collected from patients with chronic diabetic foot ulcers of which 82 isolates were obtained. The most common isolated organism was Staphylococcus aureus followed by E.coli, which correlates with the findings of our study. Eighty of the

isolates were multi drug resistant with majority being gram negative. They reported a resistance of 56.1% to Amoxicillin Clavulanic acid which is much lower than what was observed in our study. [15] Another study done by Jamatia A *et al*, a retrospective analysis of 359 consecutive pus specimens were done to evaluate the antibiotic susceptibility done by modified Kirby-Bauer methods (n=176). They reported *Staphylococcus aureus* to be the most common organism isolated which correlates with our study, followed by *Pseudomonas* spp., *Klebsiella* spp. and *Escherichia coli*. They reported a 100% resistance to amoxicillin clavulanic acid which is much higher than that present in organisms in our locality.[16] All the evidences indicate the rising levels of resistance to amoxicillin clavulanic acid calling for much judicious use of the antibiotic. Though this was a simple study, it throws light on the raising concern of antibiotic resistance being developed to a drug used to overcome resistant mechanism. This compels us to show more attention in using correct antibiotic policies as well as to increase the research on newer and more effective antibiotics. However, in our study as only the pus cultures sent for analysis to the department of Microbiology were analysed, many factors like the diabetic status of the patients, prior antibiotic use and other relevant factors that may influence resistance pattern are not accounted for. Also, significantly different susceptibility results are obtained with change in the antibiotic disc, laboratories tested, and locality.[17] Hence a more clinical correlation of the results obtained is necessary for appropriate judgement. Antibiotic drug resistance is not a new phenomenon; however the current magnitude of the problem and the speed with which the new resistant phenotypes have emerged elevates the public health importance of this issue. The societal and financial costs of treating antimicrobial resistant infections place a significant human and economic burden on society as individuals infected with drug resistant organisms are more likely to remain in hospital for a longer period of time and have poor prognosis. In addition, the scarcity of new antimicrobial agents and the lack of new agents in the drug development pipeline limit treatment options, particularly for patients with infections caused by multi drug resistant (MDR) organisms, which occur mainly in health care settings. For drug industry, it represents diminished marketability of current products.[18] As a result, it has become very important to take quick and effective steps against the emergence of antimicrobial resistance.

#### Conclusion

It has become inevitable for formulation of antibiotic policy for rational drug use, regular monitoring of progress in implementation of policies and guidelines related to antibiotic resistance, which could help in the modern day scenario of emerging resistance. Application of issues of "Antibiotic stewardship" effectively can limit the increasing antibiotic resistance as: 1) Indiscriminate use of antibiotics to be avoided by ensuring their indication, dose and duration of treatment. 2) Restrict the use of combination of antimicrobials to appropriate circumstances. 3) Restrict the drug use, e.g. limit the use of newest member of a group of antibiotics as long as the current drugs are effective. 4) Monitoring of resistance pattern constantly in a hospital or community. 5) Change the empirical drug therapy when needed. 6) Practices of infection control in hospitals to be made compulsory. Therefore our study concludes the most commonly encountered organism in pus samples to be *Staphylococcus*, followed by *E.coli*, *Pseudomonas* and *Klebsiella*. Nearly 70% of the organisms causing purulent infections were resistant to AMC, which calls for a judicious use this broad spectrum antibiotic. Of the isolates, statistically significant resistance was shown by *E.coli* followed by *Klebsiella* and *Staphylococcus*. However a clinical correlation of the results needs to be considered in choice and use of antibiotics. The

development of antibiotic resistance can be prevented by rational and judicious use of antibiotics.

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