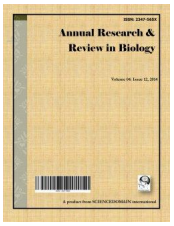

Annual Research & Review in Biology

28(3): 1-7, 2018; Article no.ARRB.41785
ISSN: 2347-565X, NLM ID: 101632869

Antibiogram Pattern Development of Pyogenic Bacteria and the Evaluation of their Multi-drug Resistance

Asma Bashir[1*], Kiran Fatima[1], Neha Farid[1] and Kashif Ali[1]

[1]Department of Biosciences, 90 and 100 Clifton, Shaheed Zulfikar Ali Bhutto Institute of Science and Technology, Block 5, Clifton 75600, Karachi, Pakistan.

Authors' contributions

This work was carried out in collaboration between all authors. Authors AB and KA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors NF and AB managed the analyses of the study. Author KF managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/41785
Editor(s):
(1) Dr. Robert Villafane, Professor, Microbiology, Alabama State University, Alabama.
(2) Dr. George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Akobi Oliver Adeyemi, Federal Medical Centre, Nigeria.
(2) B. G. Viswanath, India.
(3) Emel Banu Buyukunal, Kahramanmaras Sutcu Imam University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/26012

Original Research Article

Received 30th April 2018
Accepted 21st August 2018
Published 28th August 2018

ABSTRACT

Purpose: Different studies show consistent predictable bacterial profiles in wound infections, antibiotic resistance and capacity to adapt to a changing environment, which render the pathogens a matter of concern in hospital acquired infections. Therefore, periodical monitoring of bacterial profile and their antibiotic susceptibility pattern is essential. The objective of this study is to determine the commonly encountered pathogens in pus samples along with their antibiotic susceptibility patterns.
Methodology: Pus samples received for diagnostic microbiology were processed and identified by standard protocols at the research lab of SZABIST Biosciences Department. Antibiotic susceptibility testing was done by Kirby-Bauer Disc Diffusion method. MS Excel has been used for data compiling and analysis. Standard Deviation has been applied for the results analysis.
Results: Among the isolated organisms from pus specimens, *Staphylococcus aureus* (50%) was the most common followed by *Escherichia coli* (25%), *Klebsiella pneumonia* (9%), *Pseudomonas*

**Corresponding author: E-mail: aasma.bashir32@gmail.com;*

aeruginosa (8%), *Proteus species* (5%) and *Acinetobacter species* (3%). Quinolones (76.4%), Cephalosporins (66.4%) and Aminoglycosides (41.6%) were found to be the most effective antimicrobials in vitro, whereas Amoxicillin (17.4%), Minocycline (14.6%) and Trimethoprim-sulphamethaxazole (3%) were least effective. The resistance of organisms to antibiotics is increasing steadily as they are becoming more resistant to newer antibiotics, such as quinolones.
Conclusion: Doctors and nurses need to spread awareness of antibiotic resistance, and it is their duty to keep themselves updated with the latest antibiograms of commonly encountered pathogens, so that appropriate antibiotics may be provided for the treatment of infections.

Keywords: Antimicrobials; diseases; disinfection; resistance.

1. INTRODUCTION

Antibiotics were developed for the sole purpose of combating infection causing pathogenic organisms [1]. It does not cure the viral diseases but only bacterial diseases. Before the discovery of antibiotic, death rates were very high due to common occurrences like pneumonia, skin infections and childbirth. Antibiotics, such as the initially discovered class of antibiotics called Penicillin, brought a revolution for mankind, completely eradicating these pathogens from the body, but now an alarming increase in the development of resistance to most of the simple antibiotics has emerged as an important issue, requiring immediate actions to be taken in order to find an appropriate solution for it. With the passage of time and also depending on the location, resistance to different classes of antibiotics is being observed among pathogens [2]. One factor for this alarming increase in the trend of resistance as reported in the paper can be attributed to the inappropriate and imprudent use of antibiotics [3,4]. The infected people sometimes use the antibiotics without the consultation of a doctor, or sometimes, they even use it to cure viral diseases, this leads to several other problems. The world's most pressing health problems are due to the misuse of antibiotics. Bacteria become resistant to certain antibiotics due to two reasons: either they are genetically mutated, or they have acquired resistance from other bacteria. The resistant bacteria can spread through water or air. Some of the narrow-spectrum antibiotics that were effective earlier in treating diseases have lost their effectiveness; they have been replaced by comparatively expensive and broad-spectrum antibiotics instead [5,6]. It is necessary to measure the extent of susceptibility of commonly encountered pathogens to different antibiotics, and it is essential that their antibiograms and the knowledge of their resistance pattern be made accessible to all the doctors and nurses so that they prescribe proper and potent antibiotics to the affected patients. This paper reports about the type of pathogens isolated from pus and gives an account about the trends in resistivity to different antibiotics.

2. MATERIALS AND METHODS

2.1 Collection of Samples

To find out the aerobic bacterial agents causing different infections as well as their antibiotic sensitivity patterns, 500 samples from clinical isolates were collected. All swabs or samples of pus from skin were received in the research lab of SZABIST Biosciences Department from various hospitals in Karachi during a period of 1 year [7]. Draining/aspirated pus from an infected wound was collected either aseptically by disposable syringes in a sterilised container or by using a sterile cotton wool swab. All these samples were immediately transported to a central laboratory in sterile containers [8,9] via the hospital portering system where they were processed promptly for microbial examination [10]. The study was ethically approved.

2.2 Isolation and Identification of Bacterial Pathogens

For isolating the bacterial pathogens, all the specimens were inoculated onto the blood agar as well as on MacConkey agar plates. Cultures were aerobically incubated at 37°C overnight. The culture plates were examined for genus and majority of species after 24 hours incubation [11,12]. The growth of organisms on culture plates were identified by colony morphology, gram staining and standard biochemical tests. [13,14].

2.3 Antibiotic Sensitivity Testing

Antimicrobial susceptibility testing of the isolates was determined against the commonly used

antibiotics by Kirby-Bauer Disc Diffusion method [15] on Muller Hinton agar plates according to the Clinical and Laboratory Standards Institute (CLSI) [16]. Commercially available antibiotics discs were used, and zones of inhibition were measured after incubation [17,18]. MS Excel was used for data compiling and analysis. Standard Deviation was applied for the anaylsis of data.

3. RESULTS

From the 500 cultured samples, it was observed that 220 isolates showed significant growth. Mostly all the isolates, when tested for the bacterial strains, showed different patterns of growth of various microbes. All the microbes were found in variable percentages for distribution in the samples as shown in Fig. 1. *Staphylococcus aureus* was found the most among all the isolates giving 50% growth result, and the least observed growth was seen for *Acinetobacter* spp. giving 3% growth result for all the cultures isolates. The results for the percent distribution of other microbes were obtained as *Escherichia coli* (25%), *Klebsiella pneumoniae* (9%), *Pseudomonas aeruginosa* (8%), and Proteus spp. (5%).

After the identification of the microbes, the isolated organisms were tested for their sensitivity to various antibiotics. The tested isolates showed different percentages of sensitivity to various antibiotics. It was seen that *Staphylococcus aureus* strains are highly resistant to commonly used antibiotics like amoxycillin, minocycline, co-trimaxazole [19,20]. *Escherichia coli* followed by *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, and*

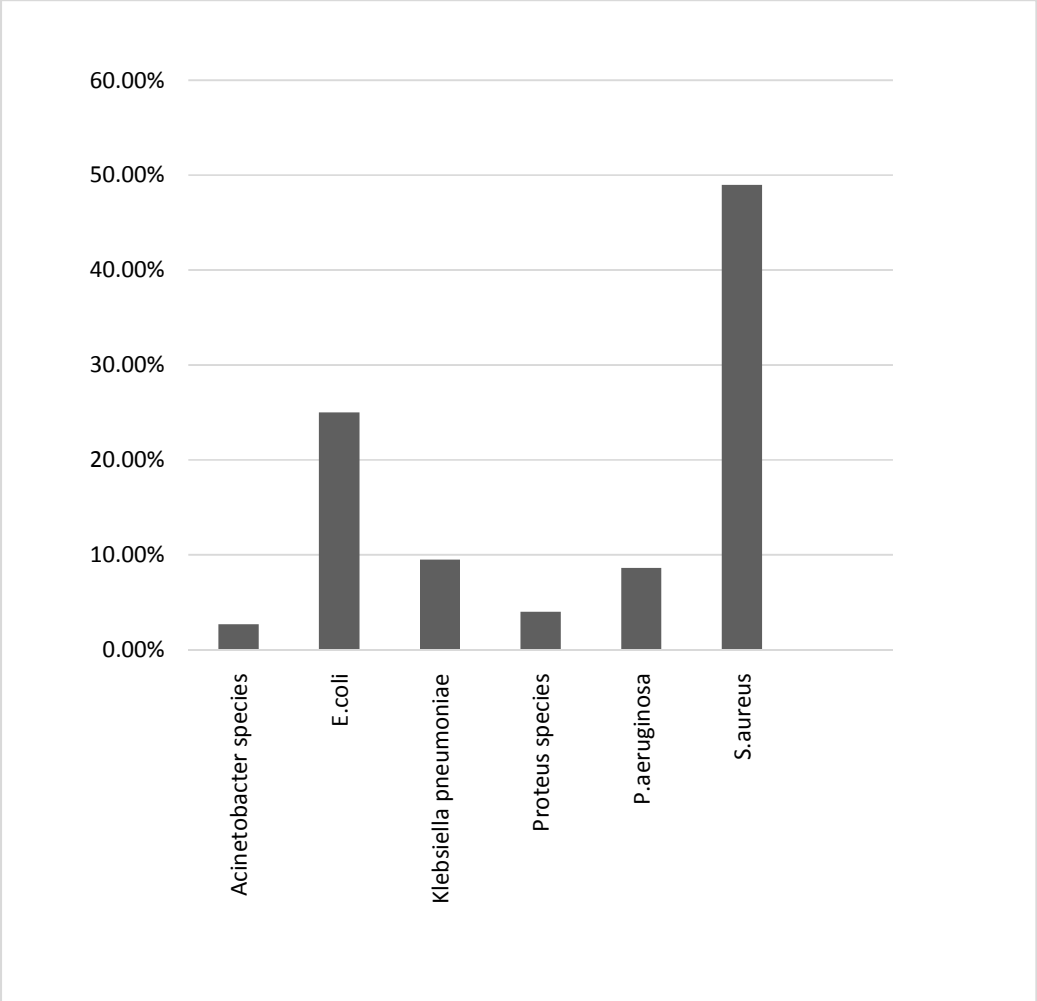

Fig. 1. Percentage distribution of isolates

Table 1. Percentage sensitivity of isolated organisms to various antibiotics

Antibiotics	*S. aureus*	*E.coli*	*K. pneumonia*	*P. aeruginosa*	*Proteus spp.*	*Standard Deviation*
	n=50	n=55	n=20	n=18	n=11	
Amoxycillin	33%	21%	0%	0%	33%	0.166222742
Augmentin	60%	30%	17%	5.80%	33%	0.20372236
Piperacillin	33%	0%	0%	0%	0%	0.147580487
Minocycline	40%	33%	0%	0%	0%	0.201444782
Erythromycin	62%	7%	0%	0%	0%	0.271145717
Clarithromycin	64%	0%	0%	0%	0%	0.286216701
Co-trimaxazole	15%	0%	0%	0%	0%	0.067082039
Gentamicin	0%	0%	0%	0%	0%	0
Tobramycin	75%	56%	77%	0%	0%	0.388497104
Amikacin	85%	77%	100%	100%	0%	0.416689333
Cefaclor	78%	50%	0%	0%	0%	0.36425266
Cefuroxime	80%	55%	33%	0%	0%	0.348898266
Cefatoxime	55%	60%	75%	0%	0%	0.354612465
Ceftizoxime	70%	87%	100%	0%	0%	0.481123685
Ceftriaxone	83%	89%	85%	75%	0%	0.374673191
Ceftazidime	77%	55%	80%	100%	0%	0.383575286
Aztreonam	10%	72%	81%	80%	83%	0.311400064
Ofloxacin	82%	78%	85%	57%	80%	0.11148991
Enoxacin	78%	80%	88%	70%	55%	0.124979998
Ciprofloxacin	67%	52%	83%	42%	0%	0.313799299

Proteus species are highly sensitive to 'Aztreonam'. Second generation Cephalosporins showed good activity (78% – 82%) against the isolates, this is in accordance with a study done before [21]. These isolates also showed a good response to 3rd generation Cephalosporins (55% - 83%) as also reported in the study [22,23]. The activity of aminoglycosides against these isolates is very well shown with previous studies showing greatest sensitivity towards amikacin than tobramycin and gentamycin. The growth was identified by observing the zone of inhibition for all the antibiotics tested against the isolated microbes. The effective antibiotics showed a clear zone of inhibition, while the antibiotics ineffective against the microbes allowed the microbes to growth to their full extent. The results of all the antibiotic susceptibility have been depicted in the Table 1. As *Acinetobacter* spp. was found in the least amount, so significant results for its antibiotic susceptibility could not be reported.

4. DISCUSSION

This study showed the predominance of *Staphylococcus aureus* in bacterial isolates as also reported in a previous study [24,25]. *Staphylococcus aureus* is found to have high versatility and resistivity among the human pathogens [26]. In hospital acquired infections and community acquired infections, *Staphylococcus aureus* is highly adaptable because of emerging resistant strains. According to present studies, the strains of *Staphylococcus aureus* show resistance to commonly used amoxicillin, minocycline, cotrimaxazole antibiotics. Sensitivity of *Staphylococcus aureus* to aminoglycosides showed no significant change of sensitivity pattern for past many years. The fact behind this unchanged condition was the indiscriminate use of aminoglycosides for *Staphylococcal* infection. A very good activity of second generation of cephalosporin i.e. 78%-82% is shown against the pathogenic isolates, the third generation cephalosporin also had effective response towards them i.e. 55%-83%. *Escherichia coli* was the second frequently encountered isolate. Other major isolates after *Escherichia coli* were *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus spp.*. These isolates have more resistance to antibiotics as compared to the reports before. Through a general observation as well as earlier studies, the isolates were seen resistant to antibiotics like amoxicillin, trimethoprim-sulphamethaxazole and minocycline that are common. The aminoglycosides activity against isolates was more sensitive towards amikacin than tobramycin and gentamycin. This showed correlation with earlier studies. Less practice of antibiotic sensitivity testing was the main reason for injudicious use of these antibiotics. Quinolone activity against pyogenic gram negative rods was also found comparable with earlier study. A high percentage of sensitivity to quinolones is observed in all bacterial strains. *Pseudomonas aeruginosa* which showed 100% sensitivity to amikacin, constituted 8% of total isolates and against which aztreonam activity was found comparable with earlier studies.

In accordance to this study and an earlier study, the resistance of organisms to antibiotics is increasing day by day and they are becoming more resistant to new antibiotics as well such as quinolones. Especially, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* species and some other *Enterobacter species* are considered the 'ESKAPE' organisms because of their ability of escaping from the effects of most of the antibiotics and show high antibiotic resistance [27,28]. One way to avoid antibiotic abuse is to provide the appropriate antibiotic as soon as the culture results are reported [29,30]. The doctors and nurses spread awareness of antibiotic resistance, therefore, it is their duty to keep themselves up to date with the latest antibiograms of commonly encountered pathogens so that appropriate antibiotic may be provided for treatment.

5. CONCLUSION

As the microbes are evolving at an incredibly fast rate, so the development of multi drug resistance is also being witnessed in the microbes. The MDR is leading to a problem on treatment of microbial infections as microbes become resistant to different antibiotics. Therefore, it is necessary to keep an assessment record of the sensitivity profiles of various microbes to different antibiotics. This will help to know the effective antibiotic against a microbe and will help in treatment and gradual eradication of infections and diseases caused by pathogenic microbes.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the author(s). The study was ethically approved.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Todar K. Antimicrobial agents in the treatment of infectious diseases. Todar's online textbook of bacteriology, University of Wisconsin, Department of Bacteriology; 2006.
   DOI: 10.4172/0974-8369.1000194
2. Sharma V, Parihar G, Sharma V, Sharma H. A study of various isolates from pus sample with their antibiogram from Jln hospital, Ajmer. IOSR-JDMS. 2015;14: 64-68.
3. Abera B, Kibret M, Mulu W. Knowledge and beliefs on antimicrobial resistance among physicians and nurses in hospitals in Amhara region, Ethiopia. BMC Pharmacol Toxicol. 2014;15:1-7.
4. Willemsen I, Groenhuijzen A, Bogaers D, Stuurman A. Appropriateness of antimicrobial therapy measured by repeated prevalence surveys. Antimicrob Agents Chemother. 2007;51(3): 864-7.
5. Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 2010;74:417-433.
6. Amado SH, Garcia FS, Blanco P, Martinez JL. Fitness costs associated with the acquisition of antibiotic resistance. Essays Biochem. 2017;61(1):37-48.
7. Shahidullah MS, Yusuf MA, Khatun Z, Aziz M. Bacteriological spectrum of different infections and their antibiogram at NICVD, Dhaka. Cardiovascular Journal. 2014;6(2): 127-132.
8. Leesik H, Ani U, Juhani A, Altaria A. Microbial pathogens of adult community-acquired pneumonia in Southern Estonia. Medicina (Kaunas). 2006;42(5):384-394.
9. Maria G, Spicuzza L, Polosa R. Antibiotics and new guidelines for the treatment of lower respiratory tract infections. Eur Respir Monogr. 2004;28:175-88.
10. Zafar A, Anwar N, Ejaz H. Bacteriology of infected wounds - A Study conducted at children's hospital Lahore. Biomedica 2008;24:71-74.
11. Nazeer HA, Shaikh KM, Kolasani BP. Aerobic bacteriology of wound infections with special reference to MRSA. J Clin Exper Res. 2014;2(1):74-79.
12. Sule A, Thanni L, Sule-Odu O, Olusanya O. Bacterial pathogens associated with infected wounds in Ogun State University Teaching Hospital, Sagamu, Nigeria. Afr J Clin Exp Microbiol. 2002;3:13-16.
13. Duggal S, Khatri PK, Parihar RS, Arora R. Antibiogram of various bacterial isolates from pus samples in a tertiary care centre in Rajasthan. Int J Sci Res. 2013;4: 1580-1584.
14. Basu S, Ramcharan PT, Bali ST, Gulati AK. Prospective, descriptive study to identify the microbiological profile of chronic wounds in outpatients. Ostomy Wound Manage. 2009;55(1):14-20.
15. Roel T, Devi M, Sahoo. Susceptibility pattern of aerobic bacterial isolates from wound swab. Indian Med Gaz. 2014;26:355-359.
16. Alka N, Priti S, Shanta N. Bacterial pathogens in urinary tract Infections and antibiotic susceptibility patterns. J Pharm Bio Sci. 2012;21:1-3.
17. Shahidullah MS, Yusuf MA, Khatun Z, Aziz M. Bacteriological spectrum of different infections and their antibiogram at NICVD, Dhaka. Cardiovasc J. 2014;6(2):127-132.
18. Rajbhandari R, Shrestha J. Bacteriological study of urinary tract infection and its antibiotic sensitivity test: A hospital based study. J Nepal Assoc Med Lab Sci. 2002; 4:26-32.
19. Sani RA, Garba SA. Antibiotic resistance profile of gram positive bacteria isolated from wound infections from areas of Niger State. Journal of Health Sciences, Department of Microbiology. 2012;2(3): 19-22.
20. Olsen MA, Nepple JJ, Riew KD. Risk factors for surgical site infection following orthopaedic spinal operations. J Bone Jt Surg Am Vol. 2008;90:62-69.
21. Swami SK, Banerjee R. Comparison of hospital-wide and age and location-stratified antibiograms of *S. aureus, E. coli,* and *S. pneumoniae*: Age- and location – stratified antibiograms. SpringerPlus. 2013;2:1-5.
22. Modi, Patel KD, Soni ST, Patel KJ. Bacteriological profile of pyogenic meningitis in tertiary care hospital, Ahmedabad. Natl J Med Res. 2012;2: 313-317.
23. Pusponegoro, Hardiono D, Oswari. Epidemiological studies of bacterial

meningitis in Jakarta and Tangerang: Preliminary report. Pediatr Infect Dis J. 1998;17(9):176-78.

24. Manjunath NS, Dayanand A, Jagannath KV, Basavarajappa KG. Prevalence of human pyogenic skin infections, bacterial pathogens and their antibiotic susceptibility profile. J Pub Health Med Res. 2013;1: 37-42.
25. Rabs N, Wieczorkiewicz SM, Costello M, Zamfirova I. Development of a urinary-Specific antibiogram for gram-negative isolates: Impact of patient risk factors on susceptibility. Am J Infect Control. 2014; 42(4):393–400.
26. Benwan K Al, Mulla A Al, Rotimi KV. Study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. J Infect Public Health. 2012;5:1-8.
27. Hout B, Oum C, Men P, Vanny V. Drug resistance in bacteria isolated from patients presenting with wounds at a non-profit surgical center in Phnom Penh, Cambodia from 2011-2013. Trop Dis Travel Med Vaccines. 2015;1:1-11.
28. Bigdeli M, Annear PL. Barriers to access and the purchasing function of health equity funds: Lessons from Cambodia. Bull W.H.O. 2009;87:260–4.
29. Zilberberg MD, Shorr AF, Micek ST, Vazquez-Guillamet. Multi-drug resistance, inappropriate initial antibiotic therapy and mortality in gram-negative severe sepsis and septic shock: A retrospective cohort study. Crit Care. 2014;18:1-13.
30. Iregui M, Ward S, Sherman G, Fraser VJ. Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. Chest. 2002;122:262-268.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/26012