Original Research Article

Bacteriological Profile & Antibiotic Resistance Pattern of Routine Clinical Isolates at a Tertiary Care Hospital in Western U.P.

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ABSTRACT

Background: Irrational & overuse of broad spectrum antibiotics such 2nd & 3rd generation cephalosporins greatly hastens the development of resistance in bacteria. Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world. The bacteriological profile & the resistance pattern vary widely in any particular area or hospital or even in different wards within a hospital. Therefore it is prudent to evaluate the prevalent bacteria causing infection & their resistance patterns which is a helpful to guide the clinicians in selecting antibiotics for various infections. Thus this prospective observational study was undertaken to document the prevalence of common bacterial isolates and their antibiotic resistance patterns of various clinical samples from patients attending the OPDs & admitted in the IPDs of the tertiary care hospital.

Methods: The present prospective observational study was carried out in the Department of Microbiology, K D Medical College Hospital & Research Centre, Mathura, Uttar Pradesh, a tertiary care centre in Western U.P, for a period of 1 year from February 2018 to January 2019. A total 1987 clinical samples (urine, blood, sputum, pus etc.) were collected and processed for culture, identification as per standard recommended procedures and antibiotic susceptibility testing were carried out on isolates as per Clinical Laboratory Standard Institute (CLSI) guidelines.

Results: Total 1987 samples were received in Department of Microbiology over a period of 1 year from February 2018 to January 2019 and 963(48.46%) were positive on culture which included 896(93.04%) bacterial and 67(6.95%) yeast isolates. E.coli, 356 (39.73%) was the predominant isolate followed by Klebsiella pneumonia 102(11.38%). The resistance pattern in E.coli to ceftazidime, amoxicillin clavulanic acid and imipenem was 52.2%, 44.3% and 9.5% respectively. Among S. aureus, 36.06% (22/61) strains were methicillin resistant.

Conclusion: Our study concluded that gram negative bacteria still remain the predominant causes in most of the clinical infections in health care settings. It further provides an insight to the clinicians into the resistance pattern of different bacterial isolates from different samples.

Key words: bacteriological profile, antibiotics, resistance pattern, clinical isolates

INTRODUCTION

Antibiotic resistance is the ability of a micro organism to withstand the effects of an antibiotic. Antibiotic resistance is a consequence of evolution via natural selection. The discovery of antibiotics in the 20th Century was a milestone for treatment of bacterial infections. The rapid emergence of resistance to antibiotics amongst pathogens generates visions of the 'potential post-antibiotic era threatening present and future medical advances'. [1]

Irrational & overuse of broad spectrum antibiotics such 2nd & 3rd generation cephalosporins greatly hastens the development of resistance in bacteria. Other factors contributing towards resistance include incorrect diagnosis, unnecessary prescription, improper use of antibiotics by patients and use of antibiotic as livestock food additives for growth promotion. As a result the bacteria are increasingly becoming resistant to commonly used antibiotics.

Antibiotic resistance among bacteria is becoming more and more serious problem throughout the world. This is particularly true in the case of members of *Enterobacteriaceae* group like *Escherichia coli* and *Klebsiella Spp* and non-fermenter group of bacteria such as *Pseudomonas Spp* and *Acinetobacter Spp*. [2,3]

The pattern of bacteria causing infections and their antibiogram vary widely from one country to another, as well as from one hospital to other and even among ICUs with one hospital. [3,4,5] Hospital antibiotic resistance patterns are commonly used to help guide empirical antimicrobial treatment and are important for detecting and monitoring trends in antimicrobial resistance.

Thus this prospective observational study was undertaken to document the prevalence of common bacterial isolates and their antibiotic resistance patterns of various clinical samples from patients attending the OPDs & admitted in the IPDs of the tertiary care hospital.

MATERIALS & METHODS

The present prospective observational study was carried out in the Department of Microbiology, K D Medical College Hospital & Research Centre, Mathura, Uttar Pradesh, a tertiary care centre in Western U.P, for a period of 1 year from February 2018 to January 2019.

The study included 1987 clinical samples for microbiological analysis that were received in the Clinical Microbiology Laboratory of Department of Microbiology.

The samples received from outpatient departments (OPDs), inpatient departments (IPDs) & various intensive care units (ICUs) included urine, pus, endotracheal secretion, exudates, body fluids, CSF.

Samples were subjected to routine Gram staining. The samples were then inoculated onto Blood agar plate, Mac Conkey agar plate and incubated aerobically at 37º C for 18-24 hours. Organisms were identified by colony morphology, Gram staining, motility & biochemical reactions as per standard bacteriological techniques [6] & identified up to species level. The culture media, reagents and chemicals used in the study were purchased from HiMedia Laboratories Private Limited, Mumbai, India.

Antibiotic susceptibility testing was done for all the isolates by the Kirby Bauer disk diffusion method in Mueller Hinton agar medium. Criteria for antimicrobial sensitivity testing were carried out as per Clinical Laboratory standard institute (CLSI). [7] The commercially available discs (Hi-media) were used. Concentration of discs used were Erythromycin (15 mcg), Vancomycin (30mcg), Cotrimoxazole (25mcg), Ciprofloxacin (5mcg), Linezolid (30mcg), Ampicillin (30mcg), Piperacillin+ Tazobactam (100/10mcg), Ceftazidime (30 mcg), Amikacin (30 mcg), Ofloxacin (5mcg), Gentamicin (10mcg) & high level (30mcg), Furazolidone (300mcg), Aztreonam (30mcg), Chloramphenicol (30mcg), and Imipenem (10mcg). Nitrofurantoin (300mcg) was used in case of urine isolates.

Methicillin resistance in *Staphylococcus aureus* (MRSA) was tested using Muller Hinton Agar with Cefoxitin disc (30mcg) by Kirby-bauer disc diffusion methods as per CLSI guidelines. [7]

Suspected extended- spectrum beta lactamases (ESBLs) producing Enterobacteriaceae were confirmed by double disk synergy test as per CLSI guidelines. [11]

Staphylococcus aureus (ATCC 25923), *E. coli* (ATCC 25922) were used as quality control throughout the study for culture and antimicrobial susceptibility testing.

The sizes of the zones of inhibition were interpreted by using the drugs recommended for all organisms by CLSI. [7]

The data was analyzed and evaluated on the basis of percentage values and the results were presented in the form of tables and figures. Microsoft excel was used for the interpretation of these results.

Statistical Analysis:

The data was analyzed on Microsoft excel sheet using percentage values as the tool. The results were presented in the form of tables and figures.

A total of 1987 samples were received in Department of Microbiology over a period of one year from February 2018 to January 2019.

The samples comprised of (42%) urine, (24%) pus, (8%) blood, (17%) sputum, (6%) body fluids, which included ascitic & pleural fluids, (2%) stool & (1%) throat swab.

Table 1: Organism-wise distribution of Gram negative bacterial isolates (n=896)

S.NO.	ORGANISM	NUMBER	PERCENTAGE
1.	Escherichia coli	356	39.73
2.	Pseudomonas spp.	78	8.70
3.	Klebsiella pneumoniae	102	11.38
4.	Acinetobacter baumannii	67	7.47
5.	Citrobacter spp.	38	4.24
6.	Enterobacter spp.	16	1.78
7.	Proteus vulgaris	47	5.24
8.	Proteus mirabilis	34	3.79

RESULTS

Table 2: Organism-wise distribution of Gram positive bacterial isolates (n=896)

S.NO.	ORGANISM	NUMBER	PERCENTAGE
1.	Staphylococcus aureus	61	6.80
2.	Coagulase Negative Staphylococcus aureus	28	3.12
3.	Enterococcus spp	69	7.70

ISOLATES	AMP	AMC	CAZ	PIT	NX	OF	CPM	IMP	CL	GEN	AK	NIT (U)	FO (U)
E.Coli (356)	289 (81.1)	158 (44.3)	186 (52.2)	-	78 (21.9)	54 (15.16)	148 (41.5)	34 (9.5)	0	51 (14.3)	32 (14.32)	23 (6.46)	0
Pseudomonas spp. (78)	-	-	39 (50)	28 (35.8)	20 (25.6)	11 (14.1)	16 (20.5)	29 (37.1)	0	10 (12.8)	-	9 (11.5)	-
Klebsiella pneumonia (102)	69 (67.6)	60 (76.9)	56 (54.9)	-		46 (58.9)	34 (33.3)	28 (27.4)	0	31 (30.3)	27 (26.4)	16 (15.6)	-
Acinetobacter baumannii (67)	60 (89.5)	-	46 (68.6)	32 (47.7)	-	35 (52.2)	34 (50.7)	42 (62.6)	0	-	18 (26.8)	-	-
Citrobacter (38)	32 (84.2)	-	24 (63.1)	6 (15.7)	12 (31.5)	13 (34.2)	7 (18.4)	8 (21)	0	-	6 (15.7)	9 (23.6)	-
Enterobacter (16)	3 (18.7)	4 (25)	7 (43.7)	-	-	6 (37.5)	2 (12.5)	1 (6.25)	0	-	2 (12.5)	1 (6.25)	-
Proteus spp.(81)	56 (69.1)	34 (41.9)	25 (30.8)	-	6 (7.4)	5 (6.2)	3 (3.7)	0	-	-	9 (23.6)	-	-

Table 3: Antibiotic resistance pattern among gram negative isolates

AMP-Ampicillin, AMC-Amoxiclav, CAZ-Ceftazidime, PIT-Piperacillin tazobactam, NX-Norfloxacin, OF-Ofloxacin, CPM- Cefipime, IMP-Imipenem, CL-Colistin, GEN-Gentamicin, AK-Amikacin, NIT-Nitrofurantoin, FO-Fosfomycin .

Table 4: Antibiotic resistance pattern among gram positive isolates

ISOLATES (n=158)	NX	FO	NIT	OF	DO	AMP	P	GEN	LZ	VA	E	COT
MSSA(39),5u	0		0	7 (17.9)	6 (15.3)	21 (53.8)	25 (64.1)	7 (17.9)	0	0	5 (12.8)	4 (10.2)
MRSA(22)	-		-	18 (81.8)	19 (86.3)	22 (100)	22 (100)	16 (72.2)	0	0	14 (63.6)	15 (68.1)
CONS(28)	-		-	5 (17.8)	4 (14.2)	14 (50)	18 (64.2)	6 (21.4)	0	0	0	0
ENTEROCOCCUS spp (69),12u	2	0	0	14 (20.2)	22 (31.8)	46 (66.6)	52 (75.3)	16 (23.1)	0	0	13 (18.8)	11 (15.9)

NX-Norfloxacin, NIT-Nitrofurantoin, FO-Fosfomycin, DO- Doxycyclin, AMP-Ampicillin, P- Penicillin, GEN- Gentamycin, LZ- Linezolid, VA- Vancomycin, E- Erythromycin, COT- Cotrimoxazole.

Out of 1987 samples processed, 963 (48.46%) yielded clinically significant growth which included 896(93.04%) bacterial and 67(6.95%) yeast isolates. Also, 82.36% (738/896) of infections were caused by gram-negative and 17.63% (158/896) by gram-positive isolates & 6.95% (67/963) were yeast infections.

Out of total 896 bacterial isolates positive, 356(39.73%) were E. coli, 78(8.70%) Pseudomonas spp., 102(11.38%) Klebsiella pneumonia, 67(7.47%) Acinetobacter baumannii, 38(4.24%) Citrobacter koseri, 16(1.78%) Enterobacter sp., 47(5.24%) Proteus vulgaris, 34(3.79%) Proteus mirabilis, 61(6.80%) Staphylococcus aureus, 69(7.70%) Enterococcus spp, 28(3.12%) Coagulase negative Staphylococcus spp.

E coli (39.73%) was the most frequently isolated organism in our study. Among E. coli, 52.2% (186/356) of isolates showed resistance to third generation cephalosporins while 44.3% (158/356) of E coli strains were resistant to amoxicillin clavulanic acid, 9.5% (34/356) of E.coli were resistant to imipenem. Klebsiella pneumonia was the next frequently isolated organism. The resistance profile of Klebsiella pneumonia was 54.9% (56/102) for ceftazidime, 76.9%(60/102) & 27.4(28/102) for amoxicillin clavulanic & imipenem respectively. Out of 288 E.coli & Klebsiella species, 43 (14.93%) were ESBL producers.
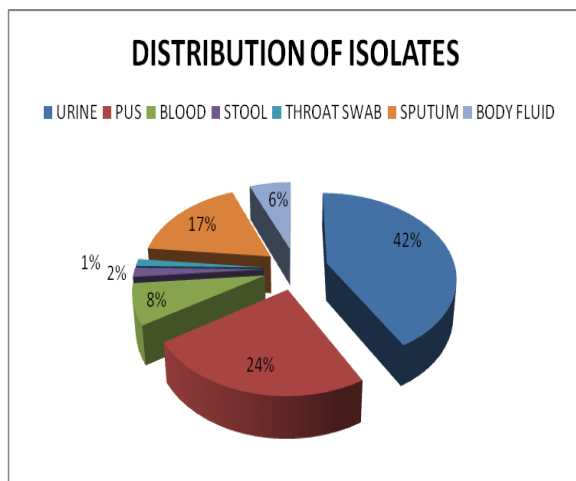
Out of 61 Staphylococcus aureus, 36.06% (22/61) strains were methicillin resistant while 63.93% (39/61) were methicillin sensitive. In case of Enterococcus spp, 31.8% (22/69) isolates were resistant to doxycycline but none were resistant to vancomycin & linezolid.
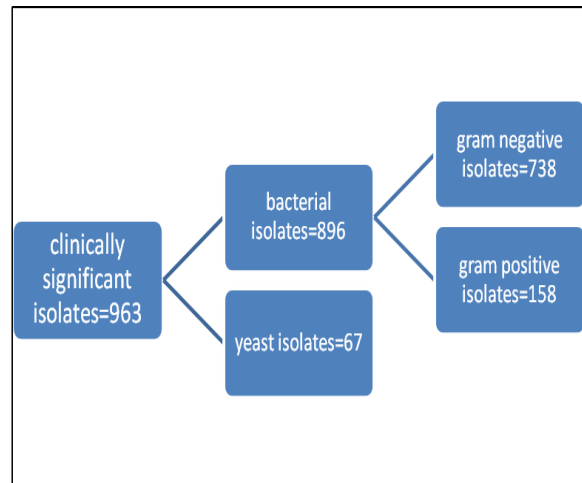

Figure 2: Distribution of clinical isolates with significant growth
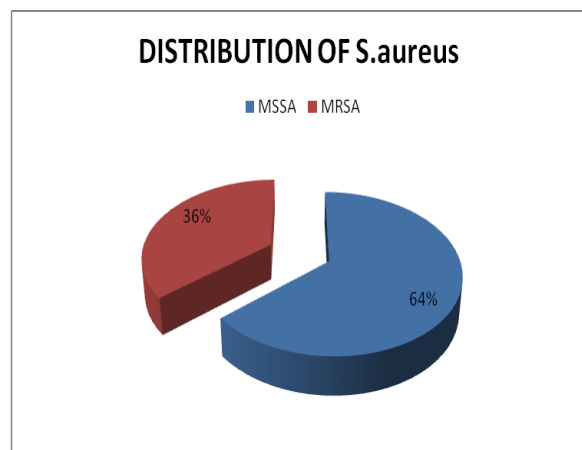

Figure 3: Distribution of MSSA & MRSA (n=61)
MSSA: Methicillin Sensitive S.aureus, MRSA: Methicillin Resistant S.aureus

DISCUSSION

The microbial pathogens, as well as their antibiotic sensitivity patterns may change from time to time and place to place. The overuse and misuse of antibiotic is leading to emergence of resistance. Hospital antibiogram are commonly used to help guide empiric antimicrobial treatment and are important component of detecting and monitoring trends in antimicrobial resistance.

Figure 1: Distribution of clinical isolates (N=1987)

Our study demonstrated that gram negative bacterial infections were most predominant infections (82.36%) at our setup while gram positive infections were only 17.63% & 6.95% were yeast infections.

In the studies conducted in mid 1990's gram-positive bacteria, particularly S. aureus and vancomycin resistant Enterococcus faecium emerged as major pathogens in abdominal and surgical site infections. [8] These trends reflected both an absolute and a proportionate increase in gram positive infections. Studies have previously shown that bacteremias due to MRSA in hospitals, increased from 11.7% in 1990 to 39.2% in 1998. [9] But gradually infections caused by MDR gram-negative bacilli have become a growing problem, with a decline in the proportion of MRSA bacteremias. [10] This transition has resulted from a number of practices that have since been implemented, which includes surveillance cultures of nasal swabs on admission to detect S. aureus colonization. Also contact and isolation precautions for those colonized with S. aureus, and the use of alcohol-based hand-washing gels have been very helpful in combating MRSA. [9]

E Coli (n=356) was the most frequently isolated organism followed by Klebsiella spp. (n=102) and Pseudomonas spp (n=78). Amongst gram negative bacilli *Escherichia coli* was dominant pathogen isolated from urine and skin & soft tissue infections whereas *Klebsiella pneumonia* was mostly isolated from lower respiratory tract infections and blood stream infection. However Gram-negative bacteria also cause infections including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis in healthcare settings. [11] Klebsiella spp., Proteus spp., Acinetobacter baumannii, Pseudomonas spp. were other important gram negative bacteria isolated from a variety of clinical samples in our study. The study showed a very high percentage of resistance among organisms to beta-lactam antibiotics, combination of beta-lactam/ beta-lactamase

inhibitors. Our study also found out that around 14.3% Enterobacteriaceae isolates were ESBL producers which is in concordance with other studies. [12]

Out of 61 Staphylococcus aureus, 36.06% (22/61) strains were methicillin resistant while 63.93% (39/61) were methicillin sensitive. Similar observations were made by Prasanth V Venniyil in a study of community-associated methicillin-resistant *Staphylococcus aureus* in patients with pyoderma who recorded the frequency of Methicillin sensitive Staphylococcus aureus (MSSA) much higher (78.12%) than Methicillin resistant Staphylococcus aureus (21.98%). [13]

In case of gram positive bacteria most of isolates especially *Staphylococcus aureus* were sensitive to vancomycin & linezolid. Resistance to ofloxacin, cotrimoxazole and erythromycin was considerably higher in MRSA than MSSA. Most of *Enterococci Spp* were 100% sensitive to vancomycin/linezolid, but had 66.6% resistance for ampicillin and 18.8% resistance was seen with erythromycin. Our sensitivity pattern was in concordance with studies carried out by many other researchers though in their study resistance pattern to vancomycin was on higher side as compared to our study. [14,15]

Similarly, some recent studies have summarized that MSSA infection still remains more prevalent despite the increasing incidence of MRSA infections. [16,17] Various authors have an opinion that as most S. aureus infections are attributable to MSSA, clinicians should be encouraged to obtain cultures from soft tissue infections before prescribing antimicrobial therapy. [16,18] The practice of assuming all cases of MRSA and treatment with vancomycin should not be encouraged. [19] Identifying MRSA from MSSA infections would assure that patients receive optimal treatment.

Our study postulated that 52.2% of E coli, 54.9% of Klebsiella spp, 50% of Pseudomonas spp, and 68.6% of Acinetobacter spp and 63.1% Citrobacter spp. were resistant to third generation

cephalosporins.

Our study observed that gram negative bacteria are acquiring resistance to multiple drugs and are increasingly resistant to most available antibiotics which are a matter of concern. It is therefore emphasized that periodic antibiograms of every hospital should be evaluated so that antimicrobial resistance in a given area can be easily monitored. Hospital antibiograms can thus be formulated from the resistance patterns & can help clinicians in empiric treatment & be more efficient in dealing with bacterial infections and to prevent the spread of drug resistant bacteria. Existing data from various studies show that there is an association between antimicrobial resistance with increase in mortality, morbidity, length of hospital stay and cost of health care. [20] Appropriate infection control measures are needed to tackle the problem as antimicrobial resistance in developing countries is increasing at an alarming rate. [21,22]

CONCLUSION

Organization & analysis of antibiotic resistance data from the laboratory database at regular intervals can provide valuable insight into the relationships between type of sample and drug-resistance in countries where such data is still scarce. Such data from laboratories when is compiled properly can contribute to national surveillance network for antibiotic resistance.

Our study concluded that gram negative bacteria still remain the predominant causes in most of the clinical infections in health care settings. E coli are the predominant organism in most of the cases. Among gram positive organisms incidence of MRSA is slowly increasing. But as prevalence of MSSA still remains high it is recommended that proper antimicrobial susceptibility testing is warranted before starting a patient on vancomycin. Antimicrobial resistance is a major challenge and antibiotics need to be tested and prescribed according to standard guidelines. Local antibiograms should be available periodically to help clinicians guide on antibiotic prescribing.

REFERENCES

1. Wise R. The world-wide threat of Resistance? (Keynote address); in proceedings of the 9th Sir Dorabji Tata Symposium. March 2008:10-11.
2. Ghafur A, Mathai D, Muruganathan A, Jayalal JA, Kant R, Chaudhary D, et al. The Chennai declaration: A roadmap to tackle the challenge of antimicrobial resistance. Indian J Cancer 2013;50:71-3.
3. Choudhury R, Panda S, Singh DV. Emergence and dissemination of antibiotic resistance: A global problem. Indian J Med Microbiol 2012;30:384-90.
4. Muto CA, Jernigan JA, Ostrowsky BE, Richet HM, Jarvis WR, Boyce JM, et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of Staphylococcus aureus and Enterococcus. Infect Control HospEpidemiol. 2003;24: 362-86.
5. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 12th ed. Elsevier -Mosby, St. Louis, Missouri 63043, USA; 2007.
6. Collee JG, Fraser AG, Marmion BP and Simmons A, Ed. Mackie and Mc Cartney Practical Medical Microbiology, 14th Ed, Churchill Livingstone: 2007.
7. CLSI Performance Standards for Antimicrobial Susceptibility Testing; Twenty Second Informational Supplement. CLSI document M100-S22. Wayne PA: Clinical & Laboratory Standards Institute.2016
8. Wade JJ, Rolando N, Hallar K, Philpott-Howard J, Casewell MW, Williams R. Bacterial and fungal infections after liver transplantation: an analysis of 284 patients. Hepatology. 1995;21:1328-1366.
9. Singh N, Paterson DL, Chang FY, Gayowski T, Squier C, Wagener MM, Marino IR. Methicillin-resistant Staphlococcus aureus: the other emerging resistant Gram-positive coccus among liver transplant recipients. Clin Infect Dis. 2000;30:332-327.
10. Singh N, Marilyn M, Obman A, Cacciarelli TV. Bacteremias in Liver Transplant Recipients: Shift Toward Gram-Negative

Bacteria as Predominant Pathogens. Liver transplant.2004;10(7).

11. CDC - Gram-negative Bacteria Infections in Healthcare Settings - HAI https://www.cdc.gov/hai/organisms/gram-negative-bacteria.html.

12. Kala Yadav ML, Ashmita Raja; bacteriological profile and antibiogram of gram negative clinical isolates from tertiary care hospital. International J res in health sci 2014;3:734-39

13. Venniyil PV, Ganguly S, Kuruvila S, Devi S. A study of community-associated. methicillin-resistant Staphylococcus aureus in patients with pyoderma. Indian Dermatol Online J. 2016;7:159-63.

14. Dash M, Padhi S, Pattnaik S, Mohanty I, Misra P. Frequency, risk factors, and antibiogram of Acinetobacter species isolated from various clinical samples in a tertiary care hospital in Odisha, India. Avicenna J Med 2013;3: 97-102.

15. Menezes GA, Harish BN, Sujatha S, Vinothini K, Parija SC. Emergence of vancomycin-intermediate Staphylococcus species in Southern India. J Med Microbiol 2008;57(Pt 7):911-2. 18

16. Mostofsky E, Lipsitch M. Is methicillin-resistant Staphylococcus aureus replacing methicillin-susceptible S. aureus? J Antimicrob Chemother. 2011;66(10):2199–2214.

17. MRSA and MSSA Remain Significant Pathogens in the NeonatalSetting.www.infectiousdiseaseadvisor.com/mrsa/addressing-mrsa...mssa-infections.../438879/.

18. Bansidhar Tarai, Poonam Das. Recurrent Challenges for Clinicians: Emergence of Methicillin-Resistant Staphylococcus aureus, Vancomycin Resistance, and Current Treatment Options. J Lab Physicians. 2013 Jul-Dec;5(2):71–78.

19. Chris Merritt, John P Haran. All purulence is local – epidemiology and management of skin and soft tissue infections in three urban emergency departments. BMC Emergency Medicine.2013.

20. Maragakis LL, Perencevich EN, Cosgrove SE. Clinical and economic burden of antimicrobial resistance. Expert Rev Anti Infect Ther. 2008 Oct;6(5):751-63.

21. Sara E. Cosgrove. The Relationship between Antimicrobial Resistance and Patient Outcomes: Mortality, Length of Hospital Stay, and Health Care Costs. Clinical Infectious Diseases.2006; 42(2): S82-S89.

22. S. Gandra, D. M Barter, R. Laxminarayan. Economic burden of antibiotic resistance: how much do we really know? Clin Microbiol Infect.2014;1-8.

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