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# Carbapenem Resistant *Pseudomonas aeruginosa, Enterobacteriaceae,* and *Acinetobacter baumannii,* Prevalence, Biochemical Identification and Clinical Characteristics in Karachi, Pakistan

Karim khan[1*], Dr. Zeba Parveen Imran[1], Faryal Anjum[2*], Mariya Yaqoob[2], Uzma Munir[1], Urooj Haroon[3]

[1] Department of Microbiology, Federal Urdu University of Arts, Science and Technology

[2] Department of Microbiology, University of Karachi

[3] Department of Chemistry, Federal Urdu University of Arts, Science and Technology

* Correspondence Author: karimkhan525@gmail.com

**ABSTRACT**

Antibiotic resistant pathogens are affecting the community and healthcare institutions all over the world. Pakistan is a developing country and resistance to drugs is the main issue and is of great importance. Current study is focused on isolation and identification of bacterial pathogens, i.e. member of *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* for the evaluation of prevalence, distribution of sensitivity and antibiogram of different antibiotics and carbapenem resistance isolates with phenotypic detection of resistant gene. Total 200 samples of different sources were collected and tested for bacterial pathogens. Out of 200 samples, 83 (41.5%) were found positive for different bacterial pathogens while 117 (58.5%) were negative. Among these 83 positive samples, Urine 43 (51.8%), Pus 22 (26.5%), Blood 8 (9.8%), Tissue 3 (3.6%), wound swab 2 (2.4%), Sputum 3 (3.6%) and HVS 2 (2.4%). *E. coli* 37 (44.6%), *Klebsiella* species 23 (27.7%), *Proteus vulgaris* 8 (9.6%), *Ps. aeruginosa* 4 (4.8%), *Acinetobacter baumannii* 4 (4.8%), *C. freundii* 2 (2.4%), *S. typhi* 2 (2.4%), *P. mirabilis* 1 (1.2%), *M .morganii* 1 (1.2%) and member of *Enterobacteriaceae* 1 (1.2%) were identified. Out of 83 (41.5%) positive samples there are 17 samples which showed resistance against Imipenem (IPM) and were further processed by phenotypic method Modified Hodge Test MHT. After Modified Hoge Test (MHT) among all these 17 samples there were only 07 (39%) positive and the remaining 11 (61%) were negative, it means that there was no gene involved in 11 samples.

**Key Words:** Modified Hodge Test (MHT), Carbapenem Resistance, *Enterobacteriaceae*, Imipenem

## 1. INTRODUCTION

Members of *Enterobacteriaceae* are gram-negative bacteria found in human gut flora. Most of them are virulent and are infectious to humans. They cause septicemia, gastrointestinal infections, pneumonia, peritonitis, infection in urinary tract and meningitis (Nordmann et al. 2012). Most bacterial infections start with inhalation of bacteria from the upper respiratory tract by altering the pharyngeal flora which is the initial step of pneumonia due to gram negative (Omer et al. 2015, Hasan et al. 2014, Johanson et al. 1969). The member of *Enterobacteriaceae* is also known to cause serious hospital acquired and communicable infections (Doyleet al. 2012). Carbapenem resitant *Enterobacteriaceae* (CRE) are a group of bacteria, highly challenging to antibiotics, and are MDR (multidrug-resistant) as well. Conversely, tolerance to carbapenem drugs among members of *Enterobacteriaceae* isolates has dramatically raised (Hu et al. 2012). Due to the overuse of antibiotics members of *Enterobacteriaceae* such as *Escherichia coli*, *Enterobacter*, *Klebsiella*, and *Salmonella* have now developed resistance to carbapenems, which were significantly adopted to deal with infections by the member of *Enterobacteriaceae* in place beta lactam drugs resistant pathogens for a long time (Temkin et al. 2014, Nordmann et al. 2012). Two major mechanisms for carbapenem resistance in *Enterobacteriaceae* are reduced outer membrane permeability by porin deficiency with the combination of an extended spectrum beta-lactamase or AmpC-type beta-lactamase and the production of beta-lactamases which are capable of hydrolyzing carbapenems (Nordmann et al. 2012, Cantón et al. 2012, Perez et al. 2013). Extended-spectrum β-lactamases (ESBLs) and AmpC-type enzymes need an additional mechanism of resistance such as efflux system over expression responsible for resistance or decrease in the uptake of antibiotics by porin deficiency (Mammeri et al., 2010, Jacoby et al., 2004, Shaikh et al., 2015). *Pseudomonas aeruginosa* is a gram-negative bacterium with unusual metabolic and physiological activities, and widely distributed in nature colonizing various environmental niches like aquatic and terrestrial plants, animals and humans as well, it can tolerate a variety of chemical and physical conditions (Klockgether et al., 2011, Silby et al., 2011). Due to many intrinsic and acquired mechanisms of resistance, spread of this pathogen is difficult to control (Breidenstein et al., 2011, Lambert 2002). *Acinetobacter* as well is another gram-negative bacterium and the most important species of this

genus is *Acinetobacter baumannii* which causes 2-10% of all Gram-negative infections in the Unites State and Europe (Fournier et al., 2006, Eveillard et al., 2010). *Ps. aeruginosa* and *Acinetobacter baumannii*, antimicrobial tolerance is due to the combination of diverse mechanisms including b-lactamase production and outer membrane modifications or a single potent resistance mechanism such as carbapenemase production (Zavascki et al., 2010, Conlan et al., 2014, Kelly et al., 2017).

## 2. MATERIALS AND METHODS

**Collection of Specimens:** The samples were collected from **Patel Hospital Karachi**. These samples include wound swabs, urine, pus, tracheal aspirate, sputum, blood, HVS and tissues for culture, both genders and patients of different age patients. A total of 200 patients' samples were taken of both hospitalized and none hospitalized patients aseptically. Samples were screened for carbapenem resistant.

**Morphological & Bio-chemical Identification**: Confirmation of members of *Enterobacteriaceae*, *ps. aeruginosa* and *Acinatobacter* species on the basis of morphological and biochemical identification was carried out by API (Analytical Profile Index). API 10 S system is used for further identification of gram-negative bacterial species. In API 10 S, 11 standardized biochemical tests and a database are used for the identification of the member of *Enterobacteriaceae* (bioMérieux, Inc, 2016).

**Antibiotic Sensitivity Testing:** Disk diffusion (Kirby-Bauer) method was used to evaluate the antibiogram pattern of isolates as it is the most common and important method for antibiotic resistance/susceptibility testing. Briefly, small filter disks having a known concentration of antibiotics were used in this method. The microorganisms to be tested are inoculated on Mueller-Hinton agar plates. The disks were placed on these inoculated Mueller-Hinton agar plates. In case of susceptibility to the antibiotic a clear zone was produced with no growth of the organism around the disk. No zone of inhibition or a relatively small zone was given by microorganisms that were resistant to that antibiotic (Kanj and Kanafani 2011).

**Recognition of Carbapenamase Enzyme (Modified Hodge Test):** The Modified Hodge Test (MHT) was used for detection of carbapenemase production in isolates. Carbapenemase production by the MHT is observed when the tested isolates produce the enzyme and allows growth of a carbapenem susceptible strain (*E. coli* ATCC 25922) towards a carbapenem disk. The positive result is characterized by a cloverleaf-like indentation.

**Identification of carbapenem resistant species:** Disk diffusion method was applied for detection of Carbapenem resistant strains of the family *Enterobacteriaceae*, *Ps.aeruginosa* and *Acinatobacter spp*. The routine antibiotic disk was put up of antibiotics (Table 4). Inhibition zones were measured against each tested antibiotic.

## 3. RESULTS & DISCUSSION

200 samples total from various sources were gathered and examined for the presence of bacteria. Samples were in vitro cultured, and the growth of microorganisms was observed based on colonial, biochemical and morphological characteristics. Only bacterial isolates listed in table 4 were further studied. Out of 200 samples, 83 (41.5%) tested positive for various bacterial infections, whereas 117 (58.5%) tested negative (Table 1). From these 83 positive samples were, 43 (51.8%) urine, 22 (26.5%) pus, 8 (9.8%) blood, 3 (3.6%) tissue, 2 (2.4%) wound swabs, 3 (3.6%) sputum, and 2 (2.4%) HVS (Table 2). The percentage of male and female patients in the positive samples was 34 (41%) and 49 (59%), respectively. Percentage of pathogenic organisms isolated from the positive clinical specimens was, *E. coli* 37(44.6%), *Proteus vulgaris* 8 (9.5%), *Klebsiella species* 23 (27.7%) *A. baumannii* 4(4.8%), *C. freundii* 2(2.4%), *Ps. aeruginosa* 4(4.8%), *S.typhi* 2 (2.4%), *P. mirabilis* 1(1.2%), *Enterobacter species* 1(1.2%) and *M. morganii* 1(1.2%) (Table 3).Out of 83 (41.5%) positive samples there are 17 samples that showed resistance against Imipenem (IPM), which were further processed by phenotypic method Modified Hodge Test (MHT) for detection of carbapenemase producing isolates. Out of 17 samples there were only 07 (39%) that showed positive results and the remaining 11 (61%) were negative implying that there was no involvement of genes in these 11 samples (Figure 1 and 2). Evaluation of carbapenemase producing bacterial isolates on genetical basis may further help in confirmatory analysis of involvement of genes in carbapenemase production. This research is only focused on the physical examination of carbapenem resistance group of gram-negative bacteria.

Based on thorough review of literature it is apparent that the trends in resistance among bacteria especially in the member of *Enterobacteriaceae* are varying from time to time. The variation in resistance pattern is associated with the region i.e. the resistance pattern in one area is different from other areas. As the resistance pattern depends upon the internal genetic makeup of the specific microorganism as well as the host health status along with living environment. Our research is therefore designed to document the frequency of carbapenem resistant in the members of *Enterobacteriaceae* species. Both community and health care institutions are under attack by antibiotic resistant pathogens worldwide (Cerqueira et al., 2017, Raro et al., 2017). Cure choices have

become narrow cause of increasing antagonism to present antimicrobials. Moreover, it has increased the seriousness of infections and increased costs. Universally, every nation is being affected by drug resistance to some levels. Anyone of any age and in any land is touched by it including developing nation like Pakistan; drug resistance is a serious issue at present. In this study a total of 200 samples were collected and examined for bacterial pathogens. The resistant pathogens to a carbapenem group of antibiotics were further processed for the phenotypic detection of carbapenamase through Modified Hodge Test (MHT). 43 (51.8%) urine, 22 (26.5%) pus, 8 (9.8%) blood, 3 (3.6%) tissue, 2 (2.4%) wound swabs, 3 (3.6%) sputum, and 2 (2.4%) HVS were among the 83 positive samples in this investigation (Table 2). In the positive samples, there were 34 (41%) and 49 (59%), respectively, male and female patients. *E. Coli* 37 (44.6%), *Proteus vulgaris* 8 (9.5%), *Klebsiella species* 23 (27.7%), *A. baumannii* 4 (4.8%), *C. freundii* 2 (2.4%), *Ps. aeruginosa* 4 (4.8%), *S. typhi* 2 (2.4%), *P. mirabilis* 1 (1.2%), *Enterobacter species* 1 (1.2%), and *M. morganii* 1 (1.2%) were the percentage of pathogenic organisms isolated from the positive clinical specimens (Table 3). 17 samples showed resistance against Imipenem (IPM), in 17 samples there were only 07 (39%) that showed positive results and remaining 11 (61%) were negative implying that there was no involvement of genes in these 11 samples (Figure 1 and 2). Our findings are relatable to another research based on the Modified Hodge test, 138 (69%) of the 200 isolates produced carbapenemase. *E. Coli* was found in 38% of the 138 MHT positive species, with *Pseudomonas aeruginosa* at 30%, *Klebsiella pneumoniae* at 17%, *Acinetobacter baumannii* at 12%, *Citrobacter diversus* at 2%, and *Enterobacter agglomerans* at 1.4% following closely behind. A straightforward technique called the Modified Hodge test may be used in a regular lab to find carbapenemases in isolates with moderate or sensitive zone diameters on disc diffusion (Ventola 2015, Miriagou et al., 2010, Amjad et al., 2011). In another study, 10.4% and 13.0% of isolates were found to be susceptible to amikacin and ciprofloxacin, respectively. Many studies have demonstrated the activity of colistin and tigecycline against carbapenemase-producing *Enterobacteriaceae* isolates (Hu et al. 2012). Prevalence of gram-negative bacteria in the human urine isolates of about more than 60% (Kolawole et al., 2009, Gadepalli et al., 2006). Another factor is used of the catheter which has been associated with infection of catheter insertion site and catheter-related infection (Rodríguez and Rello, 2007). In another study UTI was ranged in patients between 1-65 years old., the maximum number of patients with UTI were found within the age range of 20- 40 followed by the age range 1-5 and then the age range 40-65 (Romero et al., 2005, Tajbakhsh et al., 2015, Chowdhury and Parial 2015).

Table 1: Frequency distribution of different samples

| **Total Samples** | **Total Positive Samples** | **Percentage (%) of Positive Samples** | **Total Negative Samples** | **Percentage (%) of Negative Samples** |
|---|---|---|---|---|
| **200** | 83 | (41.5%) | 117 | (58.5%) |

Table 2: Frequency distribution of different Specimens

| **Specimen** | **Total Number** | **%** | **Valid %** | **Cumulative %** |
|---|---|---|---|---|
| Blood | 8 | 9.6 | 9.6 | 9.6 |
| HVS | 2 | 2.4 | 2.4 | 12.0 |
| Pus | 22 | 26.5 | 26.5 | 38.6 |
| Sputum | 3 | 3.6 | 3.6 | 45.8 |
| Tissue | 3 | 3.6 | 3.6 | 45.8 |
| Urine | 43 | 51.8 | 51.8 | 97.6 |
| Wound swab | 2 | 2.4 | 2.4 | 100.0 |
| Total | 83 | 100.0 | 100.0 | |

Table 3: Frequency distribution of isolated bacteria

| Organism | Incidences | % | Valid % | Cumulative % |
|---|---|---|---|---|
| *Acinetobacter baumannii* | 4 | 4.8 | 4.8 | 4.8 |
| *Citrobacter freundii* | 2 | 2.4 | 2.4 | 7.2 |
| *Escherichia coli* | 37 | 44.6 | 44.6 | 51.8 |
| *Enterobacter species* | 1 | 1.2 | 1.2 | 53.0 |
| *klebsiella species* | 23 | 27.7 | 27.7 | 80.7 |
| *Morganella morganii* | 1 | 1.2 | 1.2 | 81.9 |
| *Proteus mirabilis* | 1 | 1.2 | 1.2 | 83.1 |
| *Proteus vulgaris* | 8 | 9.6 | 9.6 | 92.8 |
| *Pseudomonas aeruginosa* | 4 | 4.8 | 4.8 | 97.6 |
| *Salmonella typhimurium* | 2 | 2.4 | 2.4 | 100.0 |
| Total | 83 | 100.0 | 100.0 | |

Table 4: The Antibiotic susceptibility profile of *Enterobacteriaceae, P.aeruginosa and Acinetobacter baumannii*

| S.No | Antibiotics name | Sensitive (%) | Resistant (%) |
|---|---|---|---|
| **1** | AMP (Ampicillin) | 15 | 68 |
| **2** | TZp (Piperacillin + tazobactam) | 68 | 15 |
| **3** | CAZ (Ceftazidime) | 43 | 40 |
| **4** | CRO (Ceftriaxone) | 38 | 45 |
| **5** | CFM (Cefixime) | 42 | 41 |
| **6** | FEP (Cefepime) | 44 | 39 |
| **7** | ATM (Aztreonam) | 42 | 41 |
| **8** | IPM (Imipenem) | 65 | 18 |
| **9** | CIP (Ciprofloxacin) | 40 | 43 |
| **10** | FOS (Fosfomycin) | 46 | 03 |
| **11** | SXT (Trimethoprim / sulfamethoxazole) | 25 | 58 |
| **12** | FA (Fusidic Acid) | 42 | 7 |
| **13** | SCF (Cefoperazone-Sulbactam) | 67 | 16 |
| **14** | CN (Cefalexin) | 55 | 28 |
| **15** | Ak (Amikacin) | 70 | 13 |

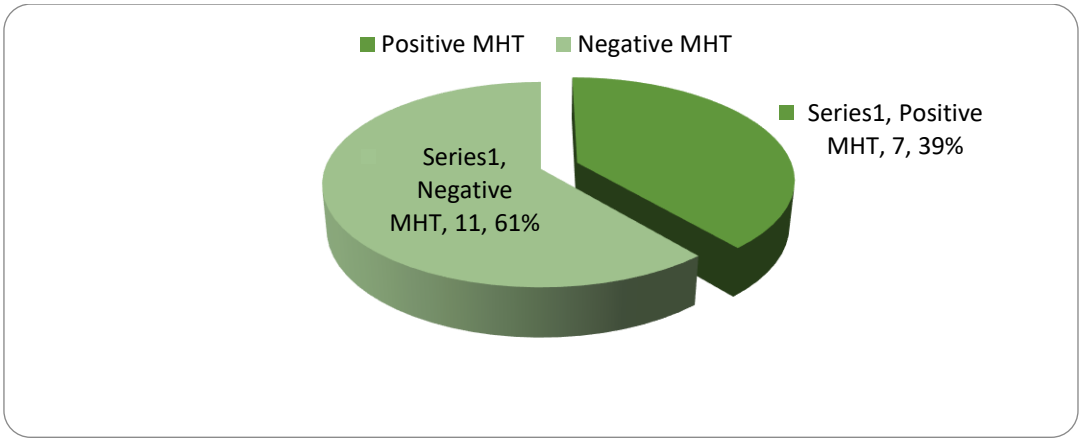


Figure 1: Percentage of microbes responsive to Modified Hodge Test (MHT)

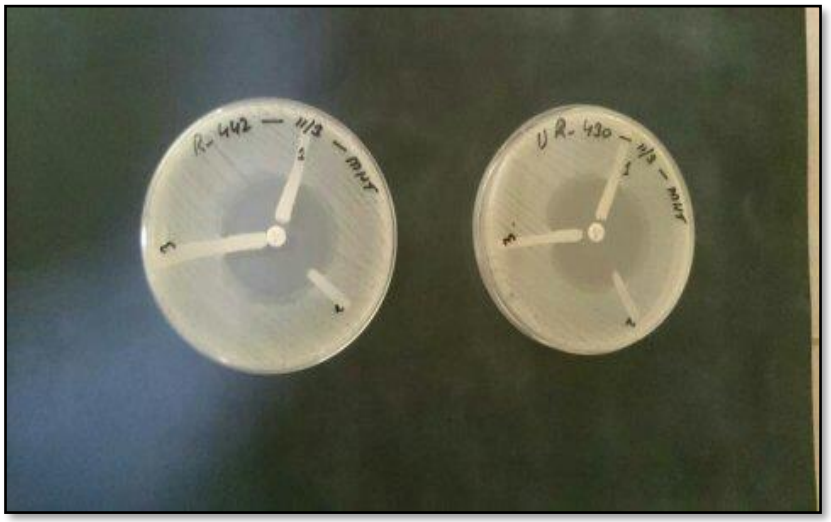

Figure 2: Petri dishes showing the MHT pattern.

## 5. REFERENCES

Amjad, A., Mirza, I. A., Abbasi, S. A., Farwa, U., Malik, N., & Zia, F. J. I. J. O. (2011). Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iranian journal of microbiology, 3(4), 189.

Breidenstein, E. B., de la Fuente-Núñez, C., & Hancock, R. E. (2011). Pseudomonas aeruginosa: all roads lead to resistance. Trends in microbiology, 19(8), 419-426..

Cantón, R., Akóva, M., Carmeli, Y., Giske, C. G., Glupczynski, Y., Gniadkowski, M., ... & Nordmann, P. (2012). Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. Clinical microbiology and infection, 18(5), 413-431.

Cerqueira, G. C., Earl, A. M., Ernst, C. M., Grad, Y. H., Dekker, J. P., Feldgarden, M., ... & Hanage, W. P. (2017). Multi-institute analysis of carbapenem resistance reveals remarkable diversity, unexplained mechanisms, and limited clonal outbreaks. Proceedings of the National Academy of Sciences, 114(5), 1135-1140.

Chowdhury, S., & Parial, R. (2015). Antibiotic susceptibility patterns of bacteria among urinary tract infection patients in Chittagong, Bangladesh. SMU Medical Journal, 114-126.

Conlan, S., Thomas, P. J., Deming, C., Park, M., Lau, A. F., Dekker, J. P., ... & Segre, J. A. (2014). Single-molecule sequencing to track plasmid diversity of hospital-associated carbapenemase-producing Enterobacteriaceae. Science translational medicine, 6(254), 254ra126-254ra126.

Doyle, D., Peirano, G., Lascols, C., Lloyd, T., Church, D. L., & Pitout, J. D. (2012). Laboratory detection of Enterobacteriaceae that produce carbapenemases. Journal of clinical microbiology, 50(12), 3877-3880.

Eveillard, M., Soltner, C., Kempf, M., Saint-André, J. P., Lemarié, C., Randrianarivelo, C., ... & Joly-Guillou, M. L. (2010). The virulence variability of different Acinetobacter baumannii strains in experimental pneumonia. Journal of Infection, 60(2), 154-161.

Fournier, P. E., Vallenet, D., Barbe, V., Audic, S., Ogata, H., Poirel, L., ... & Claverie, J. M. (2006). Comparative genomics of multidrug resistance in Acinetobacter baumannii. PLoS genetics, 2(1), e7.

Gadepalli, R., Dhawan, B., Sreenivas, V., Kapil, A., Ammini, A. C., & Chaudhry, R. (2006). A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes care, 29(8), 1727-1732.

Hasan, B., Perveen, K., Olsen, B., & Zahra, R. (2014). Emergence of carbapenem-resistant Acinetobacter baumannii in hospitals in Pakistan. Journal of medical microbiology, 63(1), 50-55.

Hu, F., Chen, S., Xu, X., Guo, Y., Liu, Y., Zhu, D., & Zhang, Y. (2012). Emergence of carbapenem-resistant clinical Enterobacteriaceae isolates from a teaching hospital in Shanghai, China. Journal of medical microbiology, 61(1), 132-136.

Jacoby, G. A., Mills, D. M., & Chow, N. (2004). Role of β-lactamases and porins in resistance to ertapenem and other β-lactams in Klebsiella pneumoniae. Antimicrobial agents and chemotherapy, 48(8), 3203-3206.

Johanson, W. G., Pierce, A. K., & Sanford, J. P. (1969). Changing pharyngeal bacterial flora of hospitalized patients: emergence of gram-negative bacilli. New England journal of medicine, 281(21), 1137-1140.

Kanj, S. S., & Kanafani, Z. A. (2011, March). Current concepts in antimicrobial therapy against resistant gram-negative organisms: extended-spectrum β-lactamase–producing enterobacteriaceae, carbapenem-resistant enterobacteriaceae, and multidrug-resistant Pseudomonas aeruginosa. In Mayo Clinic Proceedings (Vol. 86, No. 3, pp. 250-259). Elsevier.

Kelly, A. M., Mathema, B., & Larson, E. L. (2017). Carbapenem-resistant Enterobacteriaceae in the community: a scoping review. International journal of antimicrobial agents, 50(2), 127-134.

Klockgether, J., Cramer, N., Wiehlmann, L., Davenport, C. F., & Tümmler, B. (2011). Pseudomonas aeruginosa genomic structure and diversity. Frontiers in microbiology, 2, 10805.

Kolawole, A. S., Kolawole, O. M., Kandaki-Olukemi, Y. T., Babatunde, S. K., Durowade, K. A., & Kolawole, C. F. (2009). Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa state, Nigeria. International journal of medicine and medical sciences, 1(5), 163-167.

Lambert, P. (2002). Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. Journal of the royal society of medicine, 95(Suppl 41), 22.

Mammeri, H., Guillon, H., Eb, F., & Nordmann, P. (2010). Phenotypic and biochemical comparison of the carbapenem-hydrolyzing activities of five plasmid-borne AmpC β-lactamases. Antimicrobial agents and chemotherapy, 54(11), 4556-4560.

Miriagou, V., Cornaglia, G., Edelstein, M., Galani, I., Giske, C. G., Gniadkowski, M., ... & Cantón, R. (2010). Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clinical microbiology and infection, 16(2), 112-122.

Nordmann, P., Dortet, L., & Poirel, L. (2012). Carbapenem resistance in Enterobacteriaceae: here is the storm!. Trends in molecular medicine, 18(5), 263-272.

Omer, M. I., Gumaa, S. A., Hassan, A. A., Idris, K. H., Ali, O. A., Osman, M. M., ... & Khaled, M. M. (2015). Prevalence and resistance profile of Acinetobacter baumannii clinical isolates from a private hospital in Khartoum, Sudan. Am J Microbiol Res, 3(2), 76-9.

Perez, F., & Van Duin, D. (2013). Carbapenem-resistant Enterobacteriaceae: a menace to our most vulnerable patients. Cleveland Clinic journal of medicine, 80(4), 225.

Raro, O. H. F., Gallo, S. W., Ferreira, C. A. S., & Oliveira, S. D. D. (2017). Carbapenem-resistant Acinetobacter baumannii contamination in an intensive care unit. Revista da Sociedade Brasileira de Medicina Tropical, 50, 167-172.

Rodríguez, A., & Rello, J. Infection of Pulmonary Arterial and Peripheral Arterial Catheters. In Infectious Diseases in Critical Care (pp. 324-332). Berlin, Heidelberg: Springer Berlin Heidelberg..

Romero, L., Lopez, L., Rodriguez-Bano, J., Hernandez, J. R., Martinez-Martinez, L., & Pascual, A. (2005). Long-term study of the frequency of Escherichia coli and Klebsiella pneumoniae isolates producing extended-spectrum β-lactamases. Clinical microbiology and infection, 11(8), 625-631.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D., & Kamal, M. A. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi journal of biological sciences, 22(1), 90-101.

Silby, M. W., Winstanley, C., Godfrey, S. A., Levy, S. B., & Jackson, R. W. (2011). Pseudomonas genomes: diverse and adaptable. FEMS microbiology reviews, 35(4), 652-680.

Tajbakhsh, E., Tajbakhsh, S., & Khamesipour, F. (2015). Isolation and molecular detection of Gram negative bacteria causing urinary tract infection in patients referred to Shahrekord hospitals, Iran. Iranian Red Crescent Medical Journal, 17(5).

Temkin, E., Adler, A., Lerner, A., & Carmeli, Y. (2014). Carbapenem-resistant Enterobacteriaceae: biology, epidemiology, and management. Annals of the New York Academy of Sciences, 1323(1), 22-42.

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. Pharmacy and therapeutics, 40(4), 277.

Zavascki, A. P., Carvalhaes, C. G., Picao, R. C., & Gales, A. C. (2010). Multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii: resistance mechanisms and implications for therapy. Expert review of anti-infective therapy, 8(1), 71-93.