Correlation between the Antibiotic Resistance Genes and Susceptibility to Antibiotics among the Carbapenem-Resistant Gram-Negative Pathogens

Salma M. Abdelaziz 1, Khaled M. Aboshanab 1, Ibrahim S. Yahia 2,3,4, Mahmoud A. Yassien 1 and Nadia A. Hassouna 1

1 Department of Microbiology and Immunology, Faculty of Pharmacy, Ain Shams University, Organization of African Unity St., Abbasia, Cairo 11566, Egypt; salma.mustafa87@pharm.asu.edu.eg (S.M.A.); mahmoud.yassien@pharma.asu.edu.eg (M.A.Y.); nadia.hassouna@pharma.asu.edu.eg (N.A.H.)
2 Research Center for Advanced Materials Science (RCAMS), King Khalid University, Abha 61413, Saudi Arabia; ihussien@kku.edu.sa
3 Advanced Functional Materials & Optoelectronic Laboratory (AFMOL), Department of Physics, Faculty of Science, King Khalid University, Abha 9004, Saudi Arabia
4 Nanoscience Laboratory for Environmental and Bio-Medical Applications (NLEBA), Semiconductor Lab., Physics Department, Faculty of Education, Ain Shams University, Roxy, Cairo 11757, Egypt
* Correspondence: aboshanab2012@pharma.asu.edu.eg; Tel.: +20-2-2508-2595

Abstract: In this study, the correlation between the antibiotic resistance genes and antibiotic susceptibility among the carbapenem-resistant Gram-negative pathogens (CRGNPs) recovered from patients diagnosed with acute pneumonia in Egypt was found. A total of 194 isolates including Klebsiella pneumoniae (89; 46%), Escherichia coli (47; 24%) and Pseudomonas aeruginosa (58; 30%) were recovered. Of these, 34 (18%) isolates were multiple drug resistant (MDR) and carbapenem resistant. For the K. pneumoniae MDR isolates (n = 22), blaNDM (14; 64%) was the most prevalent carbapenemase, followed by blaOXA-48 (11; 50%) and blaVIM (4; 18%). A significant association (p value < 0.05) was observed between the multidrug efflux pump (AcrA) and resistance to β-lactams and the aminoglycoside acetyl transferase gene (aac-6’-Ib) gene and resistance to ciprofloxacin, azithromycin and β-lactams (except for aztreonam). For P. aeruginosa, a significant association was noticed between the presence of the blaKVE gene and the multidrug efflux pump (MexA) and resistance to fluoroquinolones, amikacin, tobramycin, co-trimoxazole and β-lactams and between the aac-6’-Ib gene and resistance to aminoglycosides. All P. aeruginosa isolates (100%) harbored the MexAB-OprM multidrug efflux pump while 86% of the K. pneumoniae isolates harbored the AcrAB-ToIC pump. Our results are of great medical importance for the guidance of healthcare practitioners for effective antibiotic prescription.

Keywords: carbapenem resistance; lower respiratory tract infections; Klebsiella pneumoniae; Pseudomonas aeruginosa; Escherichia coli; ESBL

1. Introduction

Gram-negative bacteria pose a significant treatment challenge to medical staff due to their widespread resistance to antibiotics. Klebsiella pneumoniae is a frequent human pathogen that causes many diseases, such as pneumonia, urinary tract infections and surgical wound infections, and serious life-threatening infections, such as endocarditis and sepsis. It can also cause necrotizing pneumonia and pyogenic liver abscesses [1]. It is responsible for about one-third of infections caused by all Gram-negative bacteria [2]. It is also frequently resistant to multiple antibiotics [3]. Escherichia coli, which is another member of the Enterobacteriaceae family, is the most common commensal in the gastrointestinal tract of people as well as an important pathogen. It can cause several diseases, including watery diarrhea, bloody diarrhea, urinary tract infections, acute neonatal meningitis and sepsis [4]. Pseudomonas aeruginosa is a Gram-negative bacterium that causes a myriad of diseases,
especially in critically ill and immunocompromised patients. It is a common culprit of ventilator-associated pneumonia, urinary tract infections, skin and soft tissue infections and bacteremia [5].

There is no doubt that the discovery of antibiotics at the beginning of the 20th century has saved countless lives and revolutionized modern medicine. Unfortunately, the discovery of these “magic bullets” has been inevitably accompanied by the emergence of resistant pathogens [6]. Currently, medical experts are concerned about the return to the pre-antibiotic age [7]. From the analysis of the available bacterial genomes, it was found that more than 20,000 potential resistance genes already exist in a medical database [8].

The genetic origin of drug resistance differs among drug-resistant microorganisms; it can be due to either chromosomal or mobile genetic elements [9]. Resistance acquired from mobile genetic elements, such as plasmids and transposons, is more common than from those of the chromosome. A single plasmid can carry multiple genes which encode for resistance to several drugs, thus, it spreads multiple drug resistance among microorganisms [10]. Another major problem with plasmids is that they can cross many species and genus barriers, therefore, they allow resistance to spread in bacteria that are not necessarily exposed to antibiotics [11]. Thus, devastating consequences in human health emerge from the rapid and broad dissemination of resistance determinants by plasmids [12]. In general, bacteria use three main strategies to become resistant to different antibiotics: (a) preventing the drug from reaching its target (through reduced permeability or active efflux), (b) altering the drug target and (c) inactivating the antibiotic through antibiotic destruction or modification [10]. With the advance in molecular biology techniques, the resistance genes have been extensively studied and documented [13].

Efflux pumps, which are used by almost all bacterial cells to export toxic substances from the cell metabolism, can expel antibiotics from the cell as well [14]. Five families of bacterial drug efflux pumps have been previously identified [15]. In most cases, the efflux pumps are chromosomally encoded and therefore they are not easily transferable between bacteria [16]. In resistant Gram-negative bacteria, the widely spread multidrug efflux pumps are AcrAB-TolC and MexAB-OprM, which belong to the RND superfamily. These tripartite efflux transporters were first identified and characterized in *E. coli* and *P. aeruginosa*, respectively [17]. They are known to efflux antibiotics (β-lactams, fluoroquinolones, tetracycline and chloramphenicol), heavy metals, dyes, detergents and solvents, along with many other substrates [16].

The expression of hydrolytic enzymes known as β-lactamases is the most common mechanism of bacterial resistance to β-lactams. These enzymes specifically hydrolyze the β-lactam ring, leading to an inactivated product that cannot inhibit the bacterial transpeptidases any longer [10]. There are many β-lactamases encoded on mobile genetic elements, and this leads to their increased transmission and spread. Thus, it is very common to find bacteria harboring as many as eight different β-lactamases, and each one of them specifically inactivates a unique subset of β-lactam antibiotics [18]. It is important to have reliable and easily understandable nomenclature to refer to these enzymes, as more than 4300 unique enzymes have already been identified [19]. The β-lactamases are classified into four distinct classes based on their molecular structure: classes A through D. Classes A, C and D possess a serine residue at the active site to initiate bond hydrolysis, they are thus referred to as serine β-lactamases (SBLs). In contrast, the hydrolytic action of class B β-lactamases is facilitated by one or two essential zinc ions in the active sites and therefore they are known as metallo-β-lactamases (MBLs) [18].

Notable class A enzymes include (1) TEM, which is named for a patient called Temoniera and is the first plasmid-encoded β-lactamase identified in Gram-negative bacteria; (2) sulfhydryl variant (SHV) which is an enzyme with similar activity to TEM; (3) cefotaximase (CTX-M); and (4) *K. pneumoniae* carbapenemase (KPC), which is responsible for carbapenem resistance [19]. Class A extended-spectrum β-lactamases (ESBLs) of the TEM, SHV and CTX-M families are now among the most clinically significant β-lactamases that can hydrolyze penicillins and broad-spectrum cephalosporins, as well as monobac-
tams [18]. The most clinically relevant and widespread members of class B enzymes are: (1) Verona integrin-encoded MBL (VIM); (2) imipenemase (IMP); and (3) New Delhi MBL (NDM), among others [20]. MBLs constitute the most molecularly diverse class of carbapenemases, and can hydrolyze nearly all β-lactams, except for monobactams. The class C enzymes (also referred to as AmpC enzymes) hydrolyze penicillins and cephalosporins. These enzymes are primarily chromosomally encoded; however, some have been reported to be carried on plasmids [18]. Class D SBLs include the oxacillinase (OXA) enzymes which are mainly plasmid encoded [18]. Recently, these enzymes have become increasingly important due to the ability of some members of this class to hydrolyze carbapenems along with other β-lactams, OXA-48 and related enzymes [21].

The enzymatic modification of the amino or hydroxyl groups of aminoglycosides is considered the major resistance mechanism to these antibiotics in Gram-negative and Gram-positive bacteria. This is facilitated by aminoglycoside phosphotransferases (APHs), aminoglycoside acetyltransferases (AACs) and/or aminoglycoside nucleotidyltransferases (ANTs) [10]. The structural modification of the aminoglycoside results in the inability of the modified antibiotic to bind to the target RNA due to unfavorable steric and/or electrostatic interactions [22]. Moreover, the modified variant of the enzyme, aac(6′)-Ib-cr, also exhibits a reduced quinolone susceptibility phenotype [23]. It has two amino acid changes, Trp102Arg and Asp179Tyr, which together allows the enzyme to acetylate and inactivate ciprofloxacin as well [24].

Therefore, through this research, we aim to develop an accurate local periodic report of antimicrobial resistance and to correlate the presence of certain antibiotic resistance gene(s) and susceptibility to antibiotics among the carbapenem-resistant Gram-negative pathogens (CRGNPs), particularly those conferring a multiple drug resistant (MDR) phenotype. The findings of this study will guide healthcare practitioners to more effective prescription patterns.

2. Results

The Gram-negative isolates collected in this study (n = 194) were K. pneumoniae (46%), E. coli (24%) and P. aeruginosa (30%). The antimicrobial susceptibility testing showed that the lowest resistance was observed to amikacin (15%), doxycycline (16%) and meropenem (18%). On the other hand, the highest resistance was observed to amoxicillin (79%), cefadroxil (78%), cefuroxime (78%) and cefotaxime (69%). It was found that 66.5% of the isolates (n = 129) were MDR. Table S1 (supplementary data) shows the antibiogram results of the Gram-negative isolates in this study.

The meropenem-resistant isolates were selected for further study, as they are considered critical level priority pathogens according to the WHO [25]. None of the collected E. coli isolates were resistant to meropenem. On the other hand, 22 K. pneumoniae isolates (25%) and 12 P. aeruginosa isolates (21%) were resistant to meropenem. The results of the antibiogram results of the carbapenem-resistant isolates are shown in Table S2 (supplementary data). Detailed antibiogram results and genes detected in the carbapenem-resistant K. pneumoniae and P. aeruginosa isolates are shown in Tables S3 (supplementary data) and S4 (supplementary data), respectively.

All the meropenem-resistant K. pneumoniae and P. aeruginosa isolates were resistant to cefotaxime and all of them contained one or more of the ESBL enzymes studied, however, only 16 K. pneumoniae (73%) and three P. aeruginosa (25%) isolates gave double disk synergy test (DDST). On the other hand, 19 meropenem-resistant K. pneumoniae (86%) and six meropenem-resistant P. aeruginosa (50%) harbored one or more of the carbapenemases studied, however, only 18 K. pneumoniae and three P. aeruginosa isolates gave positive modified Hodge test (MHT). Thus, the sensitivity of MHT for the K. pneumoniae isolates was almost 95% and for the P. aeruginosa isolates it was 50%. The results of the DDST and the MHT of the K. pneumoniae and P. aeruginosa isolates are shown in Tables S3 and S4 (supplementary data), respectively. Several resistance genes were studied and their results
are shown in Table 1, the number of resistance genes carried per resistant isolate is shown in Tables 2 and 3 shows different antimicrobial resistance genotypes of the CRGNPs.

| Gene          | K. pneumoniae (n = 22) n° (%) | P. aeruginosa (n = 12) n° (%) |
|---------------|-------------------------------|-------------------------------|
| blaKPC        | 0 (0)                         | 0 (0)                         |
| blaIMP        | 0 (0)                         | 0 (0)                         |
| blavIM        | 4 (18)                        | 3 (25)                        |
| blaNDM        | 14 (64)                       | 1 (8)                         |
| blaOXA        | 11 (50)                       | 3 (25)                        |
| blaCTX-M      | 15 (68)                       | 8 (67)                        |
| blaSHV        | 10 (45)                       | 11 (92)                       |
| blaTEM        | 10 (45)                       | 7 (58)                        |
| aac(6')-Ib-cr | 20 (91)                       | 10 (83)                       |
| mexA          | -                             | 12 (100)                      |
| acrA          | 19 (86)                       | -                             |

n°: number of isolates carrying the gene, %: approximate percentage.

| n° of Resistance Genes/Isolate | K. pneumoniae | P. aeruginosa | Total Isolates |
|-------------------------------|---------------|---------------|----------------|
|                               | n° | %   | n° | %   | 4° | %   |
| 7                             | 3  | 9   | 8  | 23  | 11 | 30  |
| 6                             | 8  | 23  | 10 | 29  | 18 | 45  |
| 5                             | 10 | 29  | 5  | 15  | 15 | 40  |
| 4                             | 5  | 15  | 4  | 12  | 9  | 25  |
| 3                             | 3  | 9   | 1  | 3   | 4  | 11  |
| 2                             | 1  | 3   | 1  | 3   | 2  | 6   |
| 1                             | 1  | 3   | 1  | 3   | 2  | 6   |

n°: number of isolates carrying the gene, %: approximate percentage.

| Genotype | No. | ~% |
|----------|-----|----|
| mexA/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 5    | 14 |
| acrA/blaOXA/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib or mexA/blaOXA/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 3    | 8  |
| acrA/blaNDM/blaOXA/blaCTX-M/blaSHV/aac(6’)-Ib | 2    | 6  |
| acrA/blaNDM/blaOXA/blaCTX-M/blaSHV/aac(6’)-Ib | 2    | 6  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
| acrA/blaNDM/blaCTX-M/blaSHV/blaTEM/aac(6’)-Ib | 1    | 3  |
Table 3. Cont.

| Genotype                           | No. | ≈% |
|------------------------------------|-----|----|
| acrA/blaCTX-M/aac(6’)-Ib           | 1   | 3  |
| acrA/blaNDM/blaCTX-M               | 1   | 3  |
| blaNDM/blaCTX-M/blaTEM             | 1   | 3  |
| mexA/blaVIM/blaSHV                 | 1   | 3  |
| blaOXA/bac6’-Ib                    | 1   | 3  |
| acrA/bac6’-Ib                      | 1   | 3  |
| mexA/blaVIM                        | 1   | 3  |
| acrA                                | 1   | 3  |

As shown in Table 4, statistical analysis has shown a statistically significant association between the detection of resistance genes and the phenotypic antimicrobial resistance (p value < 0.05). Calculation of the Pearson chi-square value showed a significant association between the presence of the blaSHV gene and the multidrug efflux pump, MexA, in P. aeruginosa and resistance to fluoroquinolones, amikacin, tobramycin, co-trimoxazole and the β-lactams except for aztreonam. There was also a significant association between the presence of the aac-6’-Ib gene and resistance to aminoglycosides. Among the K. pneumoniae isolates, there was a significant association between the presence of the multidrug efflux pump, AcrA, and resistance to β-lactams except for cefepime and aztreonam. There was also a significant association between the presence of the aac-6’-Ib gene and resistance to ciprofloxacin, azithromycin and the β-lactams except for aztreonam. Statistical analysis has also shown a statistically significant association between the co-existence of antibiotic resistance genes on plasmids of the same isolate, including co-existence of blaSHV/blaCTX-M, blaSHV/blaoTEM, blaCTX-M/aac-6’-Ib and blaSHV/aac-6’-Ib. Lastly, there was also a significant association between a positive MHT and resistance to meropenem.

Table 4. Statistical association between genotype and minimum inhibitory concentration (MIC) of the antibiotics and their respective p values.

| Significant Associations (Genotype and MIC of the Antibiotic) | Pearson Chi-Square |
|---------------------------------------------------------------|-------------------|
| blaSHV/amoxicillin                                           | 0.015             |
| blaSHV/co-amoxiclav                                          | 0.015             |
| blaSHV/cefadroxil                                            | 0.015             |
| blaSHV/cefuroxime                                            | 0.015             |
| blaSHV/cefotaxime                                            | 0.015             |
| blaSHV/ciprofloxacin                                         | 0.015             |
| blaSHV/levofloxacin                                          | 0.015             |
| blaSHV/amikacin                                              | 0.019             |
| blaSHV/tobramycin                                            | 0.00              |
| blaSHV/co-trimoxazole                                        | 0.049             |
| mexA/amoxicillin                                             | 0.00              |
| mexA/co-amoxiclav                                            | 0.00              |
| mexA/cefadroxil                                              | 0.00              |
| mexA/cefuroxime                                              | 0.00              |
| mexA/cefotaxime                                              | 0.00              |
| mexA/ciprofloxacin                                           | 0.00              |
| mexA/levofloxacin                                            | 0.00              |
| mexA/tobramycin                                              | 0.015             |
| mexA/co-trimoxazole                                          | 0.002             |
| aac6’-Ib/amikacin                                            | 0.040             |
Table 4. Cont.

| Significant Associations (Genotype and MIC of the Antibiotic) | Pearson Chi-Square |
|---------------------------------------------------------------|--------------------|
| aac6'-Ib/gentamicin                                           | 0.012              |
| aac6'-Ib/tobramycin                                          | 0.005              |
| blaSHV/amikacin                                               | 0.019              |
| blaSHV/tobramycin                                             | 0.000              |
| blaCTXM/gentamicin                                            | 0.015              |
| blaTEM/gentamicin                                             | 0.002              |
| acrA/amoxicillin                                              | 0.026              |
| acrA/co-amoxiclav                                             | 0.026              |
| acrA/cefadroxil                                               | 0.026              |
| acrA/cefuroxime                                               | 0.026              |
| acrA/cefotaxime                                               | 0.026              |
| acrA/meropenem                                                | 0.026              |
| aac6'-Ib/ciprofloxacin                                        | 0.012              |
| aac6'-Ib/azithromycin                                         | 0.005              |
| aac6'-Ib/amoxicillin                                          | 0.008              |
| aac6'-Ib/co-amoxiclav                                        | 0.008              |
| aac6'-Ib/cefadroxil                                           | 0.008              |
| aac6'-Ib/cefuroxime                                          | 0.008              |
| aac6'-Ib/cefotaxime                                           | 0.008              |
| aac6'-Ib/meropenem                                            | 0.008              |
| blaSHV/blaCTXM                                                 | 0.047              |
| blaSHV/blaTEM                                                  | 0.036              |
| blaCTXM/aac6'-Ib                                              | 0.027              |
| blaSHV/aac6'-Ib                                               | 0.016              |
| Modified Hodge test/meropenem                                  | 0.001              |

MIC: minimum inhibitory concentration.

3. Discussion

Lower respiratory tract infections (LRTIs) are a global health concern as they are a leading cause of morbidity and mortality worldwide [26]. A study conducted in 2017 by the Global Burden of Diseases, Injuries, and Risk Factors reported that nearly 2.56 million deaths resulted from LRTIs, making them the fifth leading cause of death for all ages [27]. More than 50% of the deaths due to LRTIs were caused by bacteria for which antibiotics are commonly prescribed for treatment [28].

Antibiotic resistance is pernicious for both the health and economic wellbeing of societies; its threat to modern medicine has been internationally recognized and profusely addressed in recent years [29]. Resistant organisms cause infections that are more difficult to treat, as they require drugs that are often less easily available, more expensive and even more toxic [30]. Reports show that the likelihood of hospitalization and the duration of hospital stay were at least twice as great for patients infected with drug-resistant strains of the same organism [31].

Gram-negative pathogens are particularly disconcerting to medical staff as they are becoming increasingly resistant to all or nearly all available antibiotic options [32]. The emergence of MDR Gram-negative bacilli has affected almost every field of medicine [33]. The most severe Gram-negative infections are commonly caused by MDR K. pneumoniae, E. coli, P. aeruginosa and Acinetobacter [30]. The carbapenem-resistant Enterobacteriaceae, which are often referred to as “nightmare bacteria”, can survive and multiply in the sink drains of healthcare facilities. Thus, they inadvertently spread to patients and to the surrounding environment through wastewater [32]. These bacteria are medically alarming as they are resistant to carbapenems which are typically reserved as a last resort treatment option against drug-resistant pathogens [30].

K. pneumoniae was the most commonly isolated pathogen in our study. It was found that 77.5% of these isolates were MDR which was much higher than the results reported by Siwakoti et al. [34] of 28%. Fortunately, this was lower than the results reported by
El-Sokkary et al. [35] of 89.6%. El-Sokkary et al. reported a similar resistance pattern to amikacin, cefuroxime, ciprofloxacin and levofloxacin. They reported much higher resistance to co-trimoxazole and co-amoxiclav. On the other hand, they reported lower resistance to cefotaxime, ceferpine and meropenem.

Comparing our results with an African systematic review [36], we found a similar prevalence of resistance to amoxicillin, co-amoxiclav, cefuroxime, amikacin, gentamicin, levofloxacin, tetracycline, co-trimoxazole and tobramycin. We observed higher resistance to meropenem, ciprofloxacin and cefotaxime. On the other hand, we observed much lower resistance to doxycycline. Comparing our results with a study carried out in China by Duan et al. [26], we found a similar prevalence of resistance to levofloxacin only. We observed higher resistance to cefepime, meropenem, amikacin, tobramycin, ciprofloxacin and co-trimoxazole. On the other hand, we observed much lower resistance to doxycycline. Comparing our results with the study reported by Singh et al. in India [37], we found a similar prevalence of resistance to meropenem, levofloxacin and co-trimoxazole. We observed much higher resistance to co-amoxiclav, ceferpine, aztreonam and ciprofloxacin. On the other hand, we observed lower resistance to cefepime, amikacin and gentamicin. Comparing our results with another Indian study [38], we observed similar resistance to amikacin. We also observed lower resistance to co-amoxiclav, ciprofloxacin and gentamicin. However, we found much higher resistance to cefotaxime.

The antimicrobial susceptibility testing of the collected E. coli isolates showed that none of them was resistant to meropenem (0%). Approximately 60% of the isolates were MDR. Fortunately, this was lower than the results reported by another Egyptian study where 87.5% of the recovered E. coli isolates were MDR [35]. Comparing our results with the above study, we also observed a lower prevalence of resistance to amikacin, co-amoxiclav and co-trimoxazole. We found a similar resistance pattern to cefuroxime, ceferpine and levofloxacin. Additionally, none of the E. coli isolates of the above study was resistant to meropenem. On the other hand, we observed higher resistance to cefotaxime and ciprofloxacin.

Comparing our results with the previously mentioned systematic review of African countries [36], we found similar prevalence of resistance to amikacin, gentamicin, tobramycin, levofloxacin and meropenem. We observed higher resistance to cefuroxime, cefotaxime, ceferpine and ciprofloxacin. On the other hand, we observed lower resistance amoxicillin, co-amoxiclav, co-trimoxazole, doxycycline and tetracycline. Comparing our results with the study reported in India by Singh et al. [37], we found similar prevalence of resistance to levofloxacin. We observed higher prevalence of resistance to co-amoxiclav, cefotaxime, aztreonam, ciprofloxacin and co-trimoxazole. On the other hand, we observed lower resistance to cefepime, meropenem, amikacin and gentamicin. Fortunately, while comparing our results with another Indian study [38], we observed much lower resistance to co-amoxiclav, amikacin, gentamicin and ciprofloxacin. However, we found higher resistance to cefotaxime and tetracycline.

P. aeruginosa usually shows resistance to multiple antibiotics, even those with considerable anti-pseudomonal activity. Therefore, it is better to treat infections caused by P. aeruginosa when guided by the susceptibility results of individual strains [35]. Approximately 55% of the isolates were MDR. Luckily, this was lower than the results reported in Egypt by El-Sokkary et al. [35], where 65.2% of the recovered P. aeruginosa isolates were MDR. Comparing our results with the above study, we also observed lower resistance to cefepime, meropenem, amikacin, ciprofloxacin and levofloxacin.

Comparing our results with the aforementioned African systematic review [36], we found a similar prevalence of resistance to amikacin, gentamicin, amoxicillin, co-amoxiclav, cefotaxime, meropenem, ciprofloxacin and levofloxacin. We observed slightly lower resistance to co-trimoxazole and much lower resistance to tetracycline. Comparing our results with the previously mentioned study in China by Duan et al. [26], we observed a similar prevalence of resistance to cefepime, amikacin, gentamicin and levofloxacin. We observed higher resistance to tobramycin and ciprofloxacin. Fortunately, we observed
lower resistance to meropenem. Comparing our results with the study reported in India by Singh et al. [37], we found a similar prevalence of resistance to aztreonam, amikacin and gentamicin. However, we observed much higher resistance to cefepime, meropenem, ciprofloxacin and levofloxacin. Fortunately, while comparing our results with another Indian study by Vijay and Dalela [38], we observed much lower resistance to all the comparable antimicrobials tested in the study: amikacin, gentamicin, co-amoxiclav, cefotaxime, ciprofloxacin and tetracycline.

Although K. pneumoniae is usually resistant to amoxicillin, research on susceptibility to amoxicillin is still carried out in several countries around the world as not all K. pneumoniae isolates produce penicillinases [36]. This African systematic review included more than 144 studies and 149,000 samples from patients all across Africa. Likewise, several recent studies tested the susceptibility of P. aeruginosa to amoxicillin, co-amoxiclav and cefotaxime as not all P. aeruginosa isolates producecephalosporinases [36,38,39].

All of our collected meropenem-resistant K. pneumoniae isolates were resistant to amoxicillin, co-amoxiclav, cefadroxil, cefuroxime and cefotaxime. Moreover, all of them (100%) were MDR. On the other hand, the lowest resistance was observed to doxycycline (4%; only one isolate was resistant). Comparing our results with a Chinese study on carbapenem-resistant K. pneumoniae [40], we observed similar resistance to cefotaxime, tobramycin, amikacin and co-trimoxazole. Fortunately, we found lower resistance to aztreonam, cefepime, ciprofloxacin, levofloxacin and gentamicin. Similarly, when comparing our isolates with another Chinese study [41], we observed similar resistance to cefuroxime, cefotaxime and levofloxacin, as well as to amikacin and gentamicin. However, we observed higher resistance to ciprofloxacin and lower resistance to co-trimoxazole. Comparing our study with a study covering 25 hospitals in China [42], we found a similar prevalence of resistance of the carbapenem-resistant K. pneumoniae isolates to cefotaxime, amikacin, tobramycin and co-trimoxazole. On the other hand, we observed lower resistance to cefepime, aztreonam, ciprofloxacin, levofloxacin and gentamicin.

All the tested meropenem-resistant P. aeruginosa isolates (100%) were resistant to amoxicillin, co-amoxiclav, cefadroxil, cefuroxime, cefotaxime, cefepime, ciprofloxacin and levofloxacin. Moreover, they were all MDR. Unfortunately, this is much higher than another study on carbapenem-resistant Pseudomonas aeruginosa; only 65% of the isolates were MDR [43]. The mechanisms of resistance of Gram-negative bacteria to carbapenems are complex; they are a result of the production of carbapenemases, a combination of porin loss or reduced expression with the production of ESBLs, or alteration of penicillin binding proteins (PBPs) at the drug action site of carbapenem [44]. Carbapenemase production is considered the most prevalent mechanism of resistance to carbapenems worldwide [45]. Carbapenem-resistant Enterobacteriaceae are designated by the CDC as nightmare bacteria, as carbapenem-resistant K. pneumoniae has a mortality rate of 40–50%, which is almost the same as that of Ebola virus, whose mortality rate is 50–60% [46].

There is a remarkable geographic variation in the distribution of different carbapenemases among carbapenem-resistant bacteria [46]. In some regions of the USA, KPCs comprise approximately 80% of the carbapenemases detected in K. pneumoniae, while MBLs are uncommonly detected in North America, with the exception of few imported cases [47]. KPCs also predominate in Italy, Portugal, Greece and China [48]. On the contrary, NDM is the most dominant carbapenemase in England [49], while OXA-48 is considered the most predominant carbapenemase detected in K. pneumoniae in Germany [49], Spain [50], Turkey [51] and Lebanon [52]. NDM, as well as OXA-48, predominate among the carbapenem-resistant bacteria in Saudi Arabia and the Gulf countries [53]. On the other hand, K. pneumoniae harboring both KPC and VIM together has been increasingly identified in Greece and France [49]. A study from Saudi Arabia and the Gulf countries [53] reported that the most frequently detected carbapenemases were OXA-48 (49%) and NDM (23%). None of the isolates produced KPC or VIM or IMP. However, Zhang et al. [42] from China reported that the most prevalent carbapenemase-producing gene in K. pneumoniae isolates was blaKPC (77%), followed by blaNDM (15%) and finally blaIMP (2%). Another study [41] re-
ported that the most prevalent gene was bla\textsubscript{OXA} (42%), followed by bla\textsubscript{NDM} (37%), followed by bla\textsubscript{KPC} (17%) and finally bla\textsubscript{IMP} (1%). A study conducted in India on carbapenem-resistant \textit{P. aeruginosa} reported that the most frequently detected carbapenemase was bla\textsubscript{VIM} (29%), followed bla\textsubscript{NDM} (28%) and finally bla\textsubscript{SIM} and bla\textsubscript{SIM} (5% each) [54].

Patients suffering from community-acquired pneumonia usually receive empirical antimicrobial therapy, while the guidelines reserve microbiological testing for severe cases [55]. Standard microbiological identification techniques, followed by antimicrobial susceptibility testing, and then followed by PCR identification of the resistance genes of concern is a tedious process that requires several days. This delay exposes the patients to the unnecessary adverse effects of the drugs, as well as extending the hospital stay for complicated cases, which increases the risk that the patients contract a hospital-acquired infection [56]. It is extremely important to implement rapid techniques that allow the identification of the causative pathogens within a few hours. This would ensure more effective antimicrobial therapy within a few hours following the diagnosis [55]. One of these techniques is the Biofire\textsuperscript{®} FilmArray\textsuperscript{®} Pneumonia Panel which accurately identifies 33 targets in sputum and bronchoalveolar lavage samples in about one hour. It is a multiplex PCR technology that contains probes for eight respiratory viruses, 18 bacteria and seven clinically relevant resistance genes (\textit{mecA}/C, bla\textsubscript{KPC}, bla\textsubscript{NDM}, bla\textsubscript{VIM}, bla\textsubscript{OXA-48-like}, bla\textsubscript{IMP} and bla\textsubscript{CTX-M}). This technology identifies the nucleic acids in the samples even if the pathogen is fastidious or the patient received prior antimicrobial therapy which would render the culture results incomprehensive [57]. Other rapid molecular diagnosis techniques include the RespiFinder\textsuperscript{®} SMART 22 FAST, the Unyvero pneumonia cartridge, the ResPlex\textsuperscript{TM} Panels, scalable target analysis routine (STAR) technology and PLEX-ID technology [58–61]. Unfortunately, these techniques are not widespread in Egyptian hospitals as they are much more expensive.

In conclusion, the results obtained in this study are of great and relevant medical importance to healthcare practitioners for effective and proper antibiotic prescription. Investment to incorporate the rapid identification techniques in Egyptian hospitals should become a medical priority to allow improved routine care.

4. Materials and Methods

4.1. Microorganisms

A total of 194 clinical Gram-negative bacterial isolates were recovered from sputum clinical specimens discharged from the microbiology laboratory at Al-Demerdash Hospital, Cairo, Egypt from patients suffering from acute lobar pneumonia according to hospital records during the period from January 2018 to February 2019. Only patients who did not receive previous antimicrobial treatment were included in the study. The isolates were identified using conventional microbiological techniques. Further confirmation of some of the results was done using the API\textsuperscript{®} 20E identification kit (bioMérieux, Lyon, France). \textit{Escherichia coli ATCC\textsuperscript{®} 25922}, \textit{E. coli ATCC\textsuperscript{®} 35218}, \textit{K. pneumoniae ATCC\textsuperscript{®} 700603} were used in the quality control of antimicrobial disk diffusion susceptibility tests. The whole study was approved by the Faculty of Pharmacy, Ain Shams University Research Ethics Committee (ENREC-ASU-Nr. 94) where both informed and written consent was obtained from patients or parents of patients after explaining the study purpose.

4.2. Antimicrobial Susceptibility Test

The antimicrobial susceptibilities, including the Kirby–Bauer disk diffusion method and minimum inhibitory concentration (MIC), were tested as recommended by the Clinical and Laboratory Standard Institute (CLSI) [62]. Disks were obtained from Oxoid\textsuperscript{®}, UK and Bioanalyse\textsuperscript{®}, Turkey. The tested antimicrobials were: amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), amoxicillin (25 µg), amoxicillin/clavulanic acid (20/10 µg), cefadroxil (30 µg), cefuroxime (30 µg), cefotaxime (30 µg), cefepime (30 µg), meropenem (10 µg), aztreonam (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), doxycycline (30 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole SXT (1.25/23.75 µg) and azithromycin (15 µg).
The MIC microdilution broth test was done in triplicate (CLSI) [62]. Isolates resistant to at least one antimicrobial agent in 3 or more antimicrobial categories are considered MDR. This is standardized international terminology proposed by the Centers for Disease Control and Prevention (CDC) and the European CDC [63].

4.3. Phenotypic Detection of ESBLs

We performed the double disk synergy test (DDST) developed by Jarlier and co-workers to detect potential ESBL producers [64]. A fresh inoculum of the potential isolate was prepared in isotonic saline to match the turbidity of 0.5 McFarland standard suspension. Then, the surface of a Mueller–Hinton agar plate was swabbed in three different directions and along the rim of the plate. Disks containing 30 µg of cefotaxime, cefepime and aztreonam were placed 20 mm apart, center to center, from a disk containing amoxicillin/clavulanic acid (20/10 µg) on the surface of the inoculated plate and incubated at 37 °C for 16 to 18 hours. The presence of ESBL was indicated by the enhancement of the inhibition zones between any of those disks towards the disk containing clavulanic acid.

4.4. Phenotypic Detection of Carbapenemases

We performed the modified Hodge test (MHT) to detect the presence of carbapenemase. Molecular detection of the carbapenemase-coding genes remains the most specific method of detection, however, it is the most expensive option and it is susceptible to false negatives if the specific carbapenemase gene present in the isolate is not targeted. The test was done according to the CLSI guidelines [62]. The plate was examined for enhanced growth of *E. coli* ATCC® 25,922 around the test isolate streak at the intersection of the streak and the inhibition zone.

4.5. Detection of Selected Resistance Genes

The CRGNPs were selected for further study as they are categorized as critical priority pathogens. This was done according to the antibiotic-resistant priority pathogens that pose the greatest threat to human health, published by the WHO [25]. The genomic DNA was extracted using a Genomic DNA Extraction Kit (Thermo Scientific, Waltham, MA, USA) and the plasmid DNA was extracted using a GeneJet Plasmid Miniprep Kit (Thermo Scientific, USA). The extracted DNA was used as the template in the polymerase chain reaction (PCR) amplification cycles. The PCR products were detected by agarose gel electrophoresis [65]. The primers (oligonucleotides) used to amplify the studied resistance genes are listed in Table 5. Some PCR products were purified and sequenced at GATC Biotech Company (Constance, Germany) through Sigma Scientific Services Company (Cairo, Egypt) by the use of an ABI 3730xl DNA Sequencer. The products were analyzed and assembled using the Staden Package program version 3 (http://staden.sourceforge.net/ (accessed on 20 November 2020)). Finally, they were submitted in the NCBI GenBank database and their corresponding accession codes were obtained. The accession codes of the genes detected in this study are shown in Table S5 (supplementary data).

| Target Gene | Primer Sequence (5’→3’) | Size (bp) | Tₐ (°C) | Reference |
|-------------|------------------------|----------|---------|-----------|
| *bla*KPC    | P<sub>f</sub> TGTCACTGTATCGCCGTC CTCAGTGTCTACAGAAACC<br>P<sub>r</sub> | 1100     | 50      | [66]      |
| *bla*IMP    | P<sub>f</sub> CTACGCCAGCAGATCTTTG AACCAGTTTTGTGCTTACC<br>P<sub>r</sub> | 587      | 50      | [67]      |
| *bla*VIM    | P<sub>f</sub> TCTACATGACCCGCGTGTC TGTGCTTTGACAACGTTC<br>P<sub>r</sub> | 748      | 50      | [68]      |
Table 5. Cont.

| Target Gene | Primer Sequence (5'→3') | Size (bp) | Tₐ (°C) | Reference |
|-------------|--------------------------|-----------|---------|-----------|
| blaNDM      | Pf: GGTTCGCGATCTGGTTTTC  
               Pr: CCGGAATGGCTCATACAGT | 621       | 50      | [69]      |
|             |                          |           |         |           |
| blaOXA      | Pf: GCGGTGTTAAGGATGAAAC  
               Pr: CATCAAAGTTCAAACCAAACCGG | 438       | 50      | [70]      |
|             |                          |           |         |           |
| blaCTX-M    | Pf: GCGTTGGCAATGGTGCGA  
               Pr: ACCCGATATCGTTGGT | 550       | 50      | [71]      |
|             |                          |           |         |           |
| blaSHV      | Pf: GGTATGCGTATATCGGCC  
               Pr: TTAGCGTTGCCAGTGC | 867       | 50      | [72]      |
|             |                          |           |         |           |
| blaTEM      | Pf: ATGAGTATCTACAGTTCC  
               Pr: CTGAGCTGTTACCAATGGCTTA | 867       | 50      | [72]      |
|             |                          |           |         |           |
| aac(6')-Ib-cr | Pf: TTAGCGATGGCTCATACAGT  
               Pr: CGTTTGGATCTTGGTGACCT | 358       | 50      | [73]      |
|             |                          |           |         |           |
| mexA        | Pf: CGACCGCGGCCGTAGGAAGCC  
               Pr: GCGTTGGATCTTGGTGACCTA | 316       | 65      |           |
|             |                          |           |         |           |
| acaR        | Pf: ATCAGCGCAGATGGTAAAA  
               Pr: CGGGTTCGGGAAAATACCC | 312       | 50      | [75]      |

4.6. Statistical Analysis

Statistical analysis of the data was performed using IBM SPSS Statistics software for Windows v.20.0 (IBM Corp., Armonk, NY, USA). Qualitative data were expressed as frequency and percentage. A chi-square test was used to compare categorical variables. All tests were two-tailed, and p-value < 0.05 was considered as statistically significant.

5. Conclusions

The results of our study highlight the extensive spread of resistant pathogens in our community. This calls for strenuous regulations to rationalize antibiotic prescription and eliminate over-the-counter antibiotic dispensing. Improved diagnostic tests to determine the etiology of LRTIs would allow more judicious use of antibiotics. This would decrease the risk of propagating antimicrobial resistance, as well as the unwanted adverse effects of antibiotics, including the development of *Clostridium difficile*.

Supplementary Materials: The following are available online at https://www.mdpi.com/2079-6382/10/3/255/s1. Table S1: Antibiogram analysis results of the total Gram-negative isolates against different tested antimicrobial agents (n = 194), Table S2: Antibiogram analysis results of the CRGNP, Table S3: Carbapenem-resistant *K. pneumoniae* isolates, Table S4: Carbapenem-resistant *P. aeruginosa* isolates, Table S5: The NCBI accession codes of the genes detected in this study.

Author Contributions: Conceptualization, S.M.A., K.M.A., I.S.Y., M.A.Y., N.A.H.; methodology, S.M.A., K.M.A., I.S.Y., M.A.Y., N.A.H.; writing—original draft preparation, S.M.A.; writing—review and editing, S.M.A., K.M.A.; supervision, M.A.Y., K.M.A., N.A.H.; statistical analysis, I.S.Y.; funding acquisition, S.M.A., K.M.A., I.S.Y., M.A.Y., N.A.H. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by postgraduate and research affairs, Ain Shams University and the Research Center for Advanced Materials Science (RCAMS) at King Khalid University (RCAMS/KKU/016-20).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Faculty of Pharmacy, Ain Shams University Research Ethics Committee (ENREC-ASU-Nr. 94).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.
Data Availability Statement: Data are available within the article and all gene sequences are available with the NCBI accession codes provided within this manuscript.

Acknowledgments: The authors express their appreciation to the Microbiology and Immunology Department, Faculty of Pharmacy, Ain Shams University for administrative and laboratory support including materials and utensils used for experiments as well as the Research Center for Advanced Materials Science (RCAMS) at King Khalid University for funding support of this work under grant number RCAMS/KKU/016-20.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Podschun, R.; Ullmann, U. Klebsiella spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. Clin. Microbiol. Rev. 1998, 11, 589–603. [CrossRef]
2. Navon-Venezia, S.; Kondratyeva, K.; Carattoli, A. Klebsiella Pneumoniae: A Major Worldwide Source and Shuttle for Antibiotic Resistance. FEMS Microbiol. Rev. 2017, 41, 252–275. [CrossRef]
3. Milt-Homens, D.; Martins, M.; Barbosa, J.; Serafim, G.; Sarmento, M.J.; Pires, R.F.; Rodrigues, V.; Bonifácio, V.D.; Pinto, S.N. Carbapenem-Resistant Klebsiella pneumoniae Clinical Isolates: In Vivo Virulence Assessment in Galleria mellonella and Potential Therapeutics by Polycationic Oligoethyleneimine. Antibiotics 2021, 10, 56. [CrossRef]
4. Abdelwahab, R.; Yasir, M.; Godfrey, R.E.; Christie, G.S.; Element, S.J.; Saville, F.; Hassan, E.A.; Abu-Faddan, N.H.; Daef, E.A.; et al. Antimicrobial Resistance and Gene Regulation in Enterohaggregative Escherichia coli from Egyptian Children with Diarrhoea: Similarities and Differences. Virulence 2021, 12, 57–74. [CrossRef] [PubMed]
5. Bassetti, M.; Vena, A.; Croxatto, A.; Righi, E.; Guery, B. How to manage Pseudomonas aeruginosa infections. Drugs Context 2018, 7, 1–18. [CrossRef] [PubMed]
6. Breijyeh, Z.; Jubeh, B.; Karaman, R. Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules 2020, 25, 1340. [CrossRef] [PubMed]
7. Aslam, B.; Wang, W.; Arshad, M.I.; Khurshid, M.; Muzammil, S.; Rasool, M.H.; Nisar, M.A.; Alvi, R.F.; Aslam, M.A.; Qamar, M.U.; et al. Antibiotic Resistance: A Rundown of a Global Crisis. Insect. Drug Resis. 2018, 11, 1645–1658. [CrossRef]
8. Davies, J.; Davies, D. Origins and Evolution of Antibiotic Resistance. Microbiol. Mol. Biol. Rev. 2010, 74, 417–433. [CrossRef]
9. Sultan, I.; Rahman, S.; Jan, A.T.; Siddiqui, M.T.; Mondal, A.H.; Haq, Q.M.R. Antibiotics, Resistome and Resistance Mechanisms: A Bacterial Perspective. Front. Microbiol. 2018, 9, 2066. [CrossRef] [PubMed]
10. Christaki, E.; Marcou, M.; Tofarides, A. Antimicrobial Resistance in Bacteria: Mechanisms, Evolution, and Persistence. J. Mol. Evol. 2020, 88, 26–40. [CrossRef]
11. Svara, F.; Rankin, D.J. The Evolution of Plasmid-Carried Antibiotic Resistance. BMC Evol. Biol. 2011, 11, 130. [CrossRef]
12. Redondo-Salvo, S.; Fernández-López, R.; Ruiz, R.; Vielva, L.; de Toro, M.; Rocha, E.P.C.; Garcia-Barriga, M.P.; de la Cruz, F. Pathways for Horizontal Gene Transfer in Bacteria Revealed by a Global Map of Their Plasmids. Nat. Commun. 2020, 11, 3602. [CrossRef]
13. Peterson, E.; Kaur, P. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. Front. Microbiol. 2018, 9, 2928. [CrossRef]
14. Abushaheen, M.A.; Fatani, A.J.; Alosaimi, M.; Mansy, W.; George, M.; Acharya, S.; Rathod, S.; Divakar, D.D.; Jhugroo, C. Antimicrobial resistance, mechanisms and its clinical significance. Dis. Mon. 2020, 66, 100971. [CrossRef]
15. Du, D.; Wang-Kan, X.; Neuberger, A.; van Veen, H.W.; Pos, K.M.; Piddock, L.J.V.; Luisi, B.F. Multidrug Efflux Pumps: Structure, Function and Regulation. Nat. Rev. Microbiol. 2018, 16, 523–539. [CrossRef] [PubMed]
16. Kumar, A.; Schweizer, H.P. Bacterial Resistance to Antibiotics: Active Efflux and Reduced Uptake. Adv. Drug Deliv. Rev. 2005, 57, 1486–1513. [CrossRef]
17. Alibert, S.; N’gompaza Diarra, J.; Hernandez, J.; Stutzmann, A.; Fouad, M.; Boyer, G.; Pages, J.-M. Multidrug Efflux Pumps and Their Role in Antibiotic and Antiseptic Resistance: A Pharmacodynamic Perspective. Expert Opin. Drug Metab. Toxicol. 2017, 13, 301–309. [CrossRef] [PubMed]
18. King, D.T.; Sobhanifar, S.; Strynadka, N.C.J. The Mechanisms of Resistance to β-Lactam Antibiotics. In Handbook of Antimicrobial Resistance; Springer: New York, NY, USA, 2017; pp. 177–201. [CrossRef]
19. Tooke, C.L.; Hinchliffe, P.; Bragginton, E.C.; Colenso, C.K.; Hirvonen, V.H.A.; Takebayashi, Y.; Spencer, J. β-Lactamases and β-Lactamase Inhibitors in the 21st Century. J. Mol. Biol. 2019, 431, 3472–3500. [CrossRef] [PubMed]
20. Mojica, M.F.; Bonomo, R.A.; Fast, W. B1-Metallo-Beta-Lactamases: Where Do We Stand? Curr Drug Targets 2016, 17, 1029–1050. [CrossRef]
21. Bush, K.; Bradford, P.A. Interplay between β-Lactamases and New β-Lactamase Inhibitors. Nat. Rev. Microbiol. 2019, 17, 295–306. [CrossRef]
22. Kotra, L.P.; Haddad, J.; Mobashery, S. Aminoglycosides: Perspectives on Mechanisms of Action and Resistance and Strategies to Counter Resistance. Antimicrob. Agents Chemother. 2000, 44, 3249–3256. [CrossRef] [PubMed]
23. Ramirez, M.S.; Tolmasky, M.E. Amikacin: Uses, Resistance, and Prospects for Inhibition. *Molecules* 2017, 22, 2267. [CrossRef] [PubMed]

24. Park, C.H.; Robicsek, A.; Jacoby, G.A.; Sahm, D.; Hooper, D.C. Prevalence in the United States of *aac(6′)-Ib-cr* Encoding a Ciprofloxacin-Modifying Enzyme. *Antimicrob. Agents Chemother.* 2006, 50, 3953–3955. [CrossRef] [PubMed]

25. World Health Organization WHO Publishes List of Bacteria for Which New Antibiotics Are Urgently Needed. Available online: https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (accessed on 15 October 2020).

26. Duan, N.; Du, J.; Huang, C.; Li, H. Microbial Distribution and Antibiotic Susceptibility of Lower Respiratory Tract Infections Patients From Pediatric Ward, Adult Respiratory Ward, and Respiratory Intensive Care Unit. *Front. Microbiol.* 2020, 11, 1480. [CrossRef] [PubMed]

27. Roth, G.A.; Abate, D.; Abate, K.H.; Abay, S.M.; Abbafati, C.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; Abdel, J.; Abdelalim, A.; et al. Global, Regional, and National Age-Sex-Specific Mortality for 282 Causes of Death in 195 Countries and Territories, 1980–2017: A Systematic Analysis for the Global Burden of Disease Study 2017. *Lancet* 2018, 392, 1736–1788. [CrossRef]

28. Troeger, C.; Blacker, B.; Khalil, I.A.; Rao, P.C.; Cao, J.; Zimsen, S.R.M.; Albertson, S.B.; Deshpande, A.; Farag, T.; Abebe, Z.; et al. Estimates of the Global, Regional, and National Morbidity, Mortality, and Aetiologies of Lower Respiratory Infections in 195 Countries, 1990–2016: A Systematic Analysis for the Global Burden of Disease Study 2016. *Lancet Infect. Dis.* 2018, 18, 1191–1210. [CrossRef]

29. Jit, M.; Ng, D.H.L.; Luangasanatip, N.; Sandman, F.; Atkins, K.E.; Robotham, J.V.; Pouwels, K.B. Quantifying the Economic Cost of Antibiotic Resistance and the Impact of Related Interventions: Rapid Methodological Review, Conceptual Framework and Recommendations for Future Studies. *BMC Med.* 2020, 18. [CrossRef]

30. Ventola, C.L. The Antibiotic Resistance Crisis. *Pharm. Ther.* 2015, 40, 277–283.

31. Li, B.; Webster, T.J. Bacteria Antibiotic Resistance: New Challenges and Opportunities for Implant-Associated Orthopaedic Infections. *J. Orthop. Res.* 2018, 36, 22. [CrossRef]

32. Solomon, S.L.; Oliver, K.B.; Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States. *Clin. Diagn. Res.* 2014, 8, 129–136. [CrossRef]

33. Golkar, Z.; Bagasra, O.; Pace, D.G. Bacteriophage Therapy: A Potential Solution for the Antibiotic Resistance Crisis. *J. Infect. Dev. Cities* 2014, 8, 173–198. [CrossRef]

34. Siwakoti, S.; Subedi, A.; Sharma, A.; Baral, R.; Bhattarai, N.R.; Khanal, B. Incidence and outcomes of multidrug-resistant gram-negative bacteria infections in intensive care unit from Nepal- a prospective cohort study. *Antimicrob. Resist. Infect. Control.* 2018, 7, 114. [CrossRef] [PubMed]

35. Roth, G.A.; Abate, D.; Abate, K.H.; Abay, S.M.; Abbafati, C.; Abbasi, N.; Abbastabar, H.; Abd-Allah, F.; Abdela, J.; Abdelalim, A.; et al. Global, Regional, and National Age-Sex-Specific Mortality for 282 Causes of Death in 195 Countries and Territories, 1980–2017: A Systematic Analysis for the Global Burden of Disease Study 2017. *Lancet* 2018, 392, 1736–1788. [CrossRef]

36. Troeger, C.; Blacker, B.; Khalil, I.A.; Rao, P.C.; Cao, J.; Zimsen, S.R.M.; Albertson, S.B.; Deshpande, A.; Farag, T.; Abebe, Z.; et al. Estimates of the Global, Regional, and National Morbidity, Mortality, and Aetiologies of Lower Respiratory Infections in 195 Countries, 1990–2016: A Systematic Analysis for the Global Burden of Disease Study 2016. *Lancet Infect. Dis.* 2018, 18, 1191–1210. [CrossRef]

37. Singh, S.; Sharma, A.; Nag, V.L. Bacterial Pathogens from Lower Respiratory Tract Infections: A Study from Western Rajasthan. *Fam. Med. Prim. Care* 2020, 18, 938–941.

38. Vianna, F.R.; Moreira, P.P.; de Brito, E.C.; Sabino, R.; de Souza, M.A.; da Siu, A.A.; da Silva, G.D.; de Oliveira, L.R.; de Souza, M.A.; de Miranda, S.G.; et al. Resistance Phenotype and Clinical Molecular Epidemiology of Carbapenem-Resistant Klebsiella Pneumoniae among Pediatric Patients in Shanghai. *Front. Pharmacol.* 2020, 11, 224. [CrossRef] [PubMed]

39. Abbas, S.; Sabir, A.U.; Khalid, N.; Sabir, S.; Khalid, S.; Haseeb, S.; Numair Khan, M.; Ajmal, W.M.; Azhar, F.; Saeed, M.T. Frequency of Extensively Drug-Resistant Gram-Negative Pathogens in a Tertiary Care Hospital in Pakistan. *Cureus* 2020, 3953–3955. [CrossRef] [PubMed]

40. Abdalalim, A.; et al. Global, Regional, and National Age-Sex-Specific Mortality for 282 Causes of Death in 195 Countries, 1990–2016: A Systematic Analysis for the Global Burden of Disease Study 2016. *Lancet Infect. Dis.* 2018, 18, 1191–1210. [CrossRef]

41. Tian, D.; Pan, F.; Wang, C.; Sun, Y.; Zhang, H. Resistance Phenotype and Clinical Molecular Epidemiology of Carbapenem-Resistant Klebsiella Pneumoniae among Pediatric Patients in Shanghai. *Infect. Drug Resist.* 2019, 11, 1935–1943. [CrossRef]

42. Zhang, Y.; Wang, Q.; Yin, Y.; Chen, H.; Jin, L.; Gu, B.; Xie, L.; Yang, C.; Ma, X.; Li, H.; et al. Epidemiology of Carbapenem-Resistant Enterobacteriaceae Infections: Report from the China CRE Network. *Antimicrob. Agents Chemother.* 2017, 62. [CrossRef]

43. Buehrle, D.J.; Shields, R.K.; Clarke, L.G.; Potoski, B.A.; Clancy, C.J.; Nguyen, M.H. Carbapenem-Resistant *Pseudomonas aeruginosa* Bacteremia: Risk Factors for Mortality and Microbiologic Treatment Failure. *Antimicrob. Agents Chemother.* 2016, 61, 61. [CrossRef] [PubMed]

44. Gao, B.; Li, X.; Yang, F.; Chen, W.; Zhao, Y.; Bai, G.; Zhang, Z. Molecular Epidemiology and Risk Factors of Ventilator-Associated Pneumonia Infection Caused by Carbapenem-Resistant Enterobacteriaceae. *Front. Pharmacol.* 2019, 10, 262. [CrossRef] [PubMed]

45. Kumudunie, W.G.M.; Wijesooriya, L.I.; Wijayasinghe, Y.S. Comparison of Four Low-Cost Carbapenemase Detection Tests and a Proposal of an Algorithm for Early Detection of Carbapenemase-Producing Enterobacteriaceae in Resource-Limited Settings. *PLoS ONE* 2021, 16, e0245290. [CrossRef] [PubMed]
46. Perez, F.; El Chakhtoura, N.G.; Papp-Walace, K.; Wilson, B.M.; Bonomo, R.A. Treatment Options for Infections Caused by Carbapenem-Resistant Enterobacteriaceae: Can We Apply “Precision Medicine” to Antimicrobial Chemotherapy? Expert Opin. Pharmacother. 2016, 17, 761–781. [CrossRef]

47. Limbago, B.M.; Rasheed, J.K.; Anderson, K.F.; Zhu, W.; Kitchel, B.; Watz, N.; Munro, S.; Gans, H.; Banaei, N.; Kallen, A.J. IMP-Producing Carbapenem-Resistant Klebsiella Pneumoniae in the United States. J. Clin. Microbiol. 2011, 49, 4239–4245. [CrossRef]

48. Mushtaq, S.; Vickers, A.; Doumith, M.; Ellington, M.J.; Woodford, N.; Livermore, D.M. Activity of β-lactam/taniborbactam (VNRX-5133) combinations against carbapenem-resistant Gram-negative bacteria. J. Antimicrob. Chemother. 2021, 76, 160–170. [CrossRef]

49. Grundmann, H.; Livermore, D.M.; Giske, C.G.; Cantón, R.; Rossolini, G.M.; Campos, J.; Vatopoulos, A.; Gniadkowski, M.; Toth, A.; Pfeifer, Y.; et al. Carbapenem-non-susceptible Enterobacteriaceae in Europe: Conclusions from a meeting of national experts. Eurosurveillance 2010, 15, 19711. [CrossRef]

50. Oteo, J.; Saez, D.; Bautista, V.; Fernández-Romero, S.; Hernández-Molina, J.M.; Pérez-Vázquez, M.; Aracil, B.; Campos, J. Carbapenemase-Producing Enterobacteriaceae in Spain in 2012. Antimicrob. Agents Chemother. 2013, 57, 6344–6347. [CrossRef]

51. Balkan, I.I.; Aygün, G.; Aydın, S.; Mutcalı, S.I.; Kara, Z.; Kuşkucu, M.; Midilli, K.; Şemen, V.; Aras, S.; Yemişen, M.; et al. Blood Stream Infections Due to OXA-48-like Carbapenemase-Producing Enterobacteriaceae: Treatment and Survival. Int. J. Infect. Dis. 2014, 26, 51–56. [CrossRef]

52. Hammoudi, D.; Ayoub Moubareck, C.; Aires, J.; Adaime, A.; Barakat, A.; Fayad, N.; Hakime, N.; Houmani, M.; Itani, T.; Najjar, Z.; et al. Countrywide Spread of OXA-48 Carbapenemase in Lebanon: Surveillance and Genetic Characterization of Carbapenem-Non-Susceptible Enterobacteriaceae in 10 Hospitals over a One-Year Period. Int. J. Infect. Dis. 2014, 29, 139–144. [CrossRef]

53. Zowawi, H.M.; Sartor, A.L.; Bakhly, H.H.; Walsh, T.R.; Al Johani, S.M.; AlJindan, R.Y.; Alfaresi, M.; Ibrahim, E.; Al-Jardani, A.; Al-Abri, S.; et al. Carbapenemase-Producing Escherichia coli and Klebsiella Pneumoniae in the Countries of the Gulf Cooperation Council: Dominance of OXA-48 and NDM Producers. Antimicrob. Agents Chemother. 2014, 58, 3085–3090. [CrossRef]

54. Verma, N.; Prahrj, A.K.; Mishra, B.; Behera, B.; Gupta, K. Detection of Carbapenemase-Producing Pseudomonas aeruginosa by Phenotypic and Genotypic Methods in a Tertiary Care Hospital of East India. J. Lab. Physicians 2019, 11, 287–291. [CrossRef]

55. Gilbert, D.N.; Leggett, J.E.; Wang, L.; Ferdosian, S.; Gelfer, G.D.; Johnston, M.L.; Footer, B.W.; Hendrickson, K.W.; Park, H.S.; White, E.E.; et al. Enhanced Detection of Community-Acquired Pneumonia Pathogens With the BioFire® Pneumonia FilmArray® Panel. Diagn. Microbiol. Infect. Dis. 2021, 99, 115246. [CrossRef] [PubMed]

56. Moffa, M.A.; Bremmer, D.N.; Carr, D.; Buchanan, C.; Shively, N.R.; Elrufay, R.; Walsh, T.L. Impact of a Multiplex Polymerase Chain Reaction Assay on the Clinical Management of Adults Undergoing a Lumbar Puncture for Suspected Community-Onset Central Nervous System Infections. Antibiotics 2020, 9, 282. [CrossRef] [PubMed]

57. The BioFire® FilmArray® Pneumonia Panel. Available online: https://www.biofiredx.com/products/the-filmarray-panels/filmarray-pneumonia/ (accessed on 20 February 2021).

58. Hattoufi, K.; Tligui, H.; Obtel, M.; El Ftouh, S.; Kharbach, A.; Barkat, A. Molecular Diagnosis of Pneumonia Using Multiplex Real-Time PCR Assay RespiFinder®SMART 22 FAST in a Group of Moroccan Infants. Available online: https://www.hindawi.com/journals/av/2020/6212643/ (accessed on 20 February 2021).

59. Luyt, C.-E.; Hattoufi, K.; Tligui, H.; Obtel, M.; El Ftouh, S.; Kharbach, A.; Barkat, A. Molecular Diagnosis of Pneumonia Using Multiplex Real-Time PCR Assay RespiFinder®SMART 22 FAST in a Group of Moroccan Infants. Available online: https://www.hindawi.com/journals/av/2020/6212643/ (accessed on 20 February 2021).

60. Luyt, C.-E.; Hémimian, G.; Bonnet, I.; Bréchet, N.; Schmidt, M.; Robert, J.; Combes, A.; Aubry, A. Usefulness of Point-of-Care Multiplex PCR to Rapidly Identify Pathogens Responsible for Ventilator-Associated Pneumonia and their Resistance to Antibiotics: An Observational Study. Crit. Care 2020, 24, 378. [CrossRef] [PubMed]

61. Hasan, M.R.; Al Mana, H.; Young, V.; Tang, P.; Thomas, E.; Tan, R.; Tilley, P. A Novel Real-Time PCR Assay Panel for Detection of Common Respiratory Pathogens in a Convenient, Strip-Tube Array Format. J. Virol. Methods 2019, 265, 1–8. [CrossRef] [PubMed]

62. Caliendo, A.M. Multiplex PCR and Emerging Technologies for the Detection of Respiratory Pathogens. Clin. Infect. Dis. 2011, 52, S326–S330. [CrossRef] [PubMed]

63. CLSI. Performance Standards for Antimicrobial Susceptibility Testing, 26th ed.; Document M100S; Clinical and Laboratory Standards Institute: Wayne, PA, USA, 2016; ISBN 1-56238-924-6.

64. Jarlier, V.; Nicolas, M.; Fournier, G.; Philippin, A. Extended Broad-Spectrum β-Lactamases Conferring Transferable Resistance to Newer β-Lactam Agents in Enterobacteriaceae: Hospital Prevalence and Susceptibility Patterns. Rev. Infect. Dis. 1988, 10, 867–878. [CrossRef]

65. Sambrook, J.J.; Russell, D.D.W. Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press: New York, NY, USA, 2001; ISBN 0-87969-577-3.

66. Yigit, H.; Queenan, A.M.; Anderson, G.J.; Domenech-Sanchez, A.; Biddle, J.W.; Steward, C.D.; Alberti, S.; Bush, K.; Tenover, F.C. Novel Carbapenem-Hydrolyzing Beta-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of Klebsiella Pneumoniae. Antimicrob. Agents Chemother. 2001, 45, 1151–1161. [CrossRef]
67. Senda, K.; Arakawa, Y.; Ichiyama, S.; Nakashima, K.; Ito, H.; Ohsuka, S.; Shimokata, K.; Kato, N.; Ohta, M. PCR Detection of Metallo-Beta-Lactamase Gene (BlalMP) in Gram-Negative Rods Resistant to Broad-Spectrum Beta-Lactams. *J. Clin. Microbiol.* 1996, 34, 2909–2913. [CrossRef]

68. Poirel, L.; Naas, T.; Nicolas, D.; Collet, L.; Bellais, S.; Cavallo, J.-D.; Nordmann, P. Characterization of VIM-2, a Carbapenem-Hydrolyzing Metallo-β-Lactamase and Its Plasmid- and Integron-Borne Gene from a *Pseudomonas aeruginosa* Clinical Isolate in France. *Antimicrob. Agents Chemother.* 2000, 44, 891–897. [CrossRef] [PubMed]

69. Nordmann, P.; Poirel, L.; Carrière, A.; Toleman, M.A.; Walsh, T.R. How To Detect NDM-1 Producers. *J. Clin. Microbiol.* 2011, 49, 718–721. [CrossRef] [PubMed]

70. Poirel, L.; Walsh, T.R.; Cuvillier, V.; Nordmann, P. Multiplex PCR for Detection of Acquired Carbapenemase Genes. *Diagn. Microbiol. Infect. Dis.* 2011, 70, 119–123. [CrossRef] [PubMed]

71. Bonnet, R.; Dutour, C.; Sampaio, J.L.; Chanal, C.; Sirot, D.; Labia, R.; De Champs, C.; Sirot, J. Novel Cefotaximase (CTX-M-16) with Increased Catalytic Efficiency Due to Substitution Asp-240 →Gly. *Antimicrob. Agents Chemother.* 2001, 45, 2269–2275. [CrossRef] [PubMed]

72. Rasheed, J.K.; Jay, C.; Metchock, B.; Berkowitz, F.; Weigel, L.; Crellin, J.; Steward, C.; Hill, B.; Medeiros, A.A.; Tenover, F.C. Evolution of Extended-Spectrum β-Lactam Resistance (SHV-8) in a Strain of *Escherichia coli* during Multiple Episodes of Bacteremia. *Antimicrob. Agents Chemother.* 1997, 41, 647–653. [CrossRef]

73. Hamed, S.M.; Aboshanab, K.M.A.; Elkhatib, W.F.; Ashour, M.S. Aminoglycoside Resistance Patterns of Certain Gram Negative Uropathogens Recovered from Hospitalized Egyptian Patients. *Br. Microbiol. Res. J.* 2013, 3, 678–691. [CrossRef]

74. Dumas, J.-L.; van Delden, C.; Perron, K.; Köhler, T. Analysis of Antibiotic Resistance Gene Expression in *Pseudomonas aeruginosa* by Quantitative Real-Time-PCR. *FEMS Microbiol. Lett.* 2006, 254, 217–225. [CrossRef]

75. Wasfi, R.; Elkhatib, W.F.; Ashour, H.M. Molecular Typing and Virulence Analysis of Multidrug Resistant *Klebsiella Pneumoniae* Clinical Isolates Recovered from Egyptian Hospitals. *Sci. Rep.* 2016, 6, srep38929. [CrossRef] [PubMed]