

The Five Years Surveillance and Trends of Antibiotic Resistance in Some Common Gram Negative Bacteria at the Vietnam Military Hospital

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Abstract

The study is to identify trends and levels of antibiotic resistance of some common Gram-negative strains over time. The samples were collected from Vietnam Military Hospital 103 between 2014 and 2019. A total of 405 *Acinetobacter baumannii*, 528 *Pseudomonas aeruginosa*, 741 *Escherichia coli*, and 352 *Klebsiella pneumoniae* strains were identified and antimicrobial susceptibility was by Vitek system and Etest method. The multi-drug resistance (MDR) was major proportion of four common bacteria. In particular, there is a tendency to shift from MDR to Extended drug resistance (XDR) or possibly Pan drug resistant (pPDR). *A. baumannii* had the highest level of antibiotic resistance, namely, carbapenem (61.5% - 82.5%) and cephalosporin (72.7% - 88.7%). *P. aeruginosa* resisted most of commonly antibiotics, ranging from 50% to 70%. *E. coli* had a high resistance with antibiotics like ampicillin (87.2% - 97.6%) and the 3rd generation cephalosporins (up to 79.6%). *K. pneumoniae* resisted carbapenem from 14.7% to 44.4%, and other antibiotics with the higher rate of 40%. The collected data will be a prerequisite for further studies on mechanisms and factors related to antibiotic resistant bacteria, in order to find out a rational and effective using strategy of antibiotics.

Keywords

Gram-Negative Bacteria, Antibiotic Resistance, Multi-Drug Resistance

1. Introduction

The invention of antibiotics is an immense achievement in the treatment of in-

fectious diseases. However, the widespread use of antibiotics in the past decades has led to the emergence of multi-drug resistant bacteria. It caused a difficulty in infection treatment, influenced on the cost and days of treatment. It is a risk of facing global health sector [1].

Recognizing the importance of antibiotic-resistance of bacteria, global scientists and Vietnamese ones continuously research with a hope of reducing and controlling this problem. In February 2017, the World Health Organization (WHO) released a list of 12 strains of antibiotic-resistant bacteria that should be a top priority in researching and discovering new antibiotics [2]. Gram-negative bacterial strains are primary concern because of their potential to cause hospital infections and drug resistant level. Among the agents of Gram-negative bacteria, four common bacteria *A. baumannii*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* are the most mentioned species studies. Because they are not only the causes of serious infections such as sepsis, pneumonia, urinary infections and hospital infections but also the agents that are resistant to many common antibiotics [3]. Unsurprisingly, most of the mentioned strains have appeared in Vietnam. The concern is that these strains have resisted many different antibiotics and increased resistant levels over time [4] [5].

Gram-negative bacteria have been always mentioned by many studies, because their antibiotic resistance has changed over time and geographic area [6]. It is necessary to regularly monitor and study the trends as well as the level of resistance of bacteria. The aim for the clinicians has a reasonable and effective strategy in use and store antibiotics in different areas in order to improve treatment quality and limit transmission of multi-resistant strains. In addition, the results also provided extra data to finalize the global report of antibiotic resistant bacteria.

In fact, there is a great difficulty in choosing antibiotics for treatment. So, this study focuses on showing kinds of antibiotics that the common bacteria are increasing their resistance and/or sensitive in recent years.

2. Materials and Methods

2.1. Clinical Samples, Identification and Antimicrobial Susceptibility Tests of Gram-Negative Bacteria

Clinical specimens were collected from patients at the military hospital in Vietnam during 2014-2019. The isolation techniques were conducted according to WHO standards and specialized microbiological guidance of Vietnam Ministry of Health [7] [8]. The species were identified using the Vitek 2 GN ID card. Antimicrobial susceptibility testing of isolates was performed on the Vitek 2 Antimicrobial Susceptibility Test (bioMérieux, France) [9], determined the minimum inhibitory concentration of antibiotics (MIC) by Etest (bioMérieux, France) method [10]. Results of antimicrobial susceptibility tested were interpreted according to Clinical and Laboratory Standards Institute guidelines, updated every year [11]. The quality of the identification procedure was controlled

by using the *Enterobacter homachchei* ATCC 700324 strain. The strains of *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used to control the quality of antibiotic sensitivity tests.

2.2. Statistical Analyses

The data collected during the research period of the project is processed according to medical statistical methods, using WHONET 2019 antibiotic management and analysis software. Comparative testing using EpiCal 2000 software, the p values < 0.05 was considered statistically significant.

3. Results

3.1. Characteristics of Drug Resistant Trends

For all four common Gram-negative bacteria, the rates of multi-drug bacteria were higher than those of resistant bacteria under 3 antibiotic groups ($p < 0.05$). In particular, the rate of multi-resistant of *A. baumannii* tended to increase gradually over the years of research (Figure 1).

The multi-drug resistant (MDR) strains were major proportion of the 4 species of common bacteria. In particular, there is a tendency to shift from MDR to XDR (Extended drug resistance, non-susceptible in all but ≤ 2 categories) or possibly PDR (Pan drug resistant, non-susceptible to all antimicrobial listed) in the studied bacteria. The resistant *A. baumannii* were almost XDR or pPDR. The rates of them fluctuated from 95% to 100% over the years. The trend of increasing XDR or PDR strains was also evident in *P. aeruginosa* at 67.86% in 2014 and gradually reached to 90.32% in 2019 ($p = 0.02$) (Figure 2). We could not test with all of antibiotic groups, so they did not affirm which strains PDR was, and only called it pPDR.

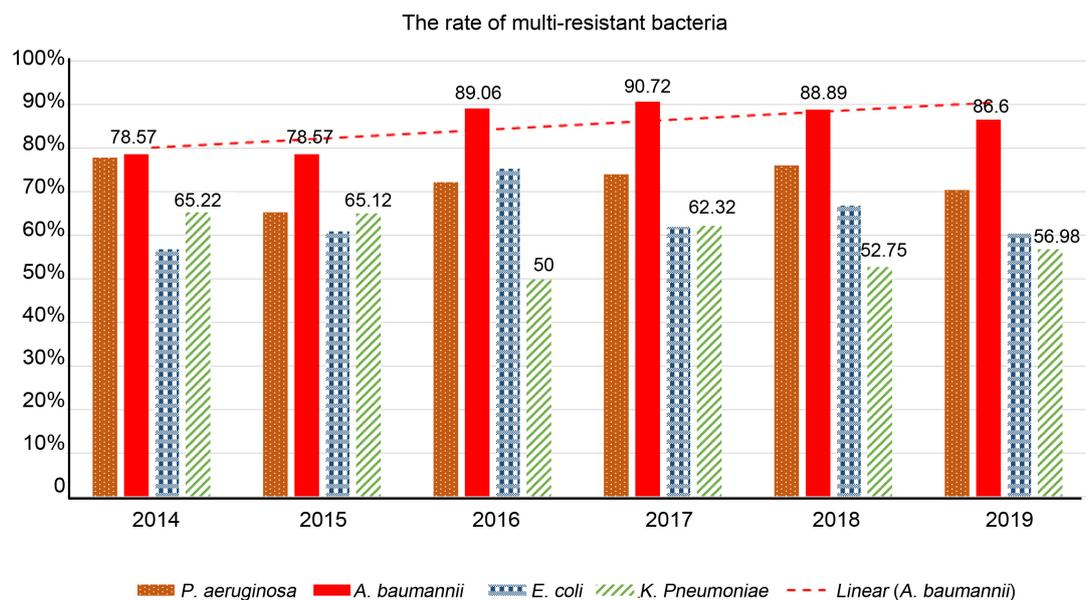


Figure 1. Distribution of multi-resistant bacteria over time.

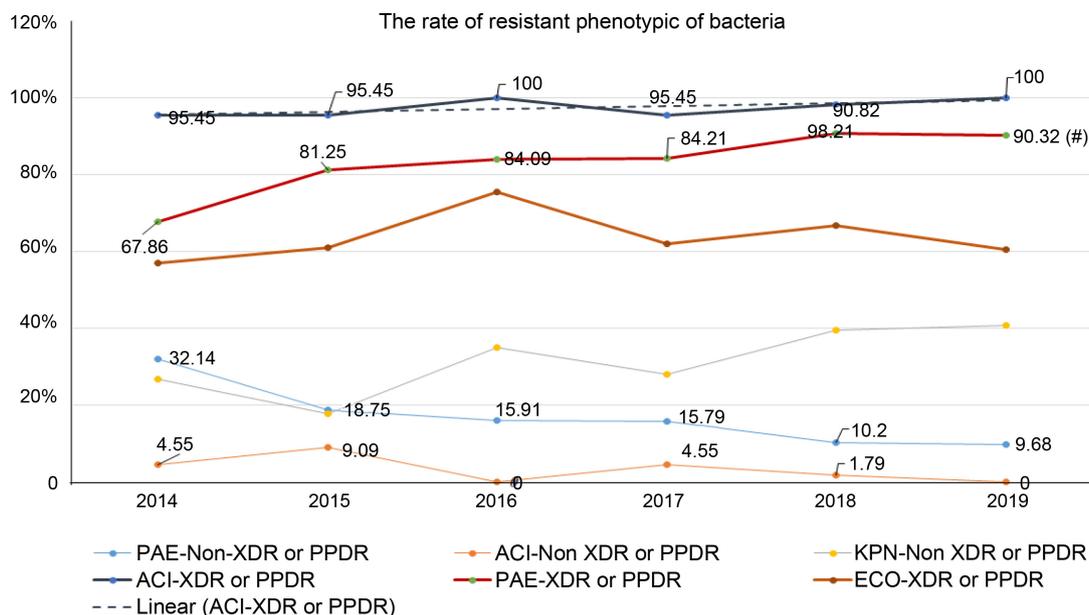


Figure 2. Distribution of MDR (Non - XDR or pPDR bacteria) and XDR or pPDR (possible PDR) bacteria on total multidrug - resistant bacteria over time. #A statistically significant result ($p = 0.02$) between XDR and PDR during 2014-2019.

3.2. The Level of Antibiotic Resistance of Some Gram-Negative Bacteria Isolated

A. baumannii was resistant with almost common antibiotics and tended to increase over time. Between 2017 and 2019, the antibiotic resistant rates of *A. baumannii* were over 80% (Figure 3).

The rate of resistance with imipenem and meropenem in 2014 was 61.5% and 70.8%. In 2019, both of rates increased to 81.8%. Amikacin and co-trimoxazole were still susceptible with *A. baumannii*. The level of resistance tended to decrease over time, but the overall resistant rates with these were quite high, at 30.0% and 37.4% respectively. Figure 4 indicated that the typical level of antibiotic resistance in *P. aeruginosa* was high (>50%) and tended to increase over time ($p < 0.05$). This bacterium was resistant with quinolones at a very high rate: levofloxacin 67.4% and ciprofloxacin 66.6%. The carbapenem antibiotics such as imipenem and meropenem had resistant rates at 57.3% and 59.7%, respectively. Resistance of *P. aeruginosa* with other antibiotics such as ceftazidime (52.0%), cefepime (48.7%), ticarcillin (68.2%), aztreonam (60.9%), and tobramycin (58.4%) was shown. Two antibiotics had effects on *P. aeruginosa* were amikacin (20.9% - 40.9%) and piperacillin (15% - 39.4%). However, the antibiotic resistant level of bacterium with these antibiotics was increasing over time, especially with a continuous increase from 2017 to 2019 (Figure 4).

E. coli had a high resistance to antibiotics, such as ampicillin (>80%), cephalosporin group (up to 79.7%), quinolone and flouroquinolone (range from 40.9% to 72.6%), co-trimoxazole (75.8%). When considering the trend of changing resistant level by year, *E. coli* tended to increase resistance to 3rd

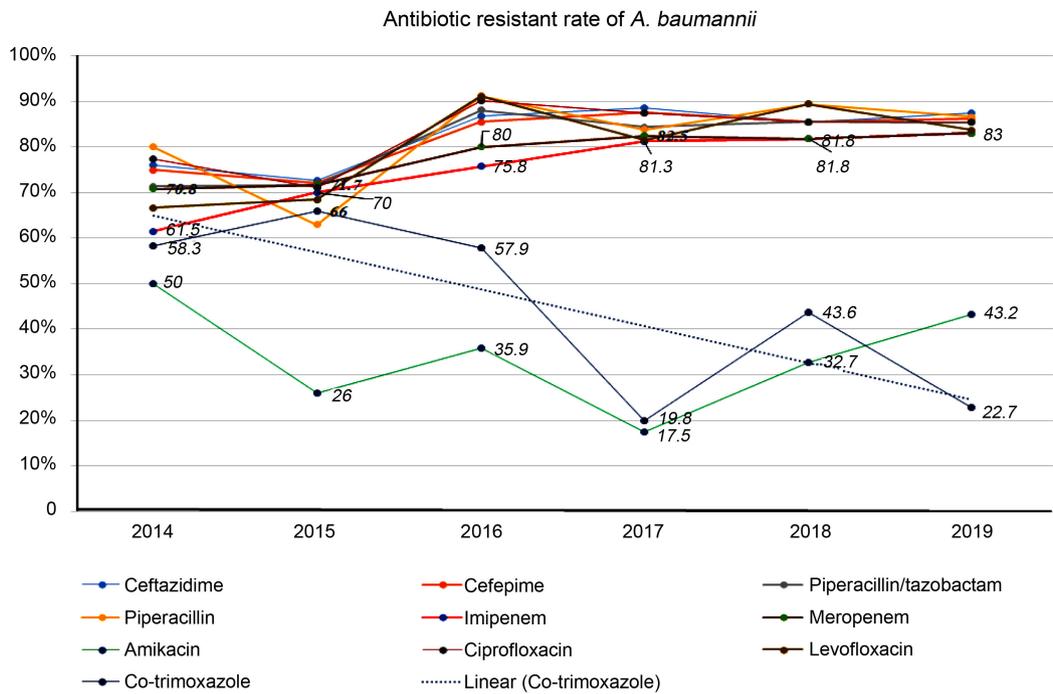


Figure 3. The resistant level of *A. baumannii* over time.

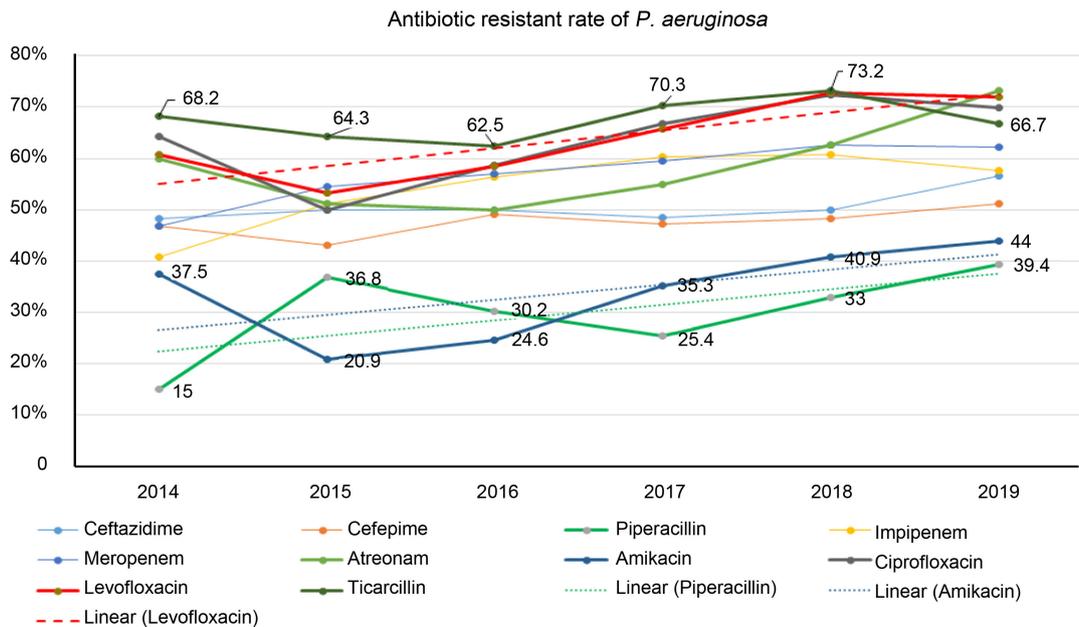


Figure 4. The resistant level of *P. aeruginosa* over time.

generation cephalosporins and quinolones (norfloxacin and ciprofloxacin) ($p < 0.05$). However cefepim, there was a slight decrease in the resistant rates but the difference between annual rates was not significant ($p > 0.05$) (Figure 5).

Klebsiella pneumoniae was resistant to many antibiotics such as cephalosporins, quinolones, aminoglycosides, and co-trimoxazole with the ratios from 25.8% to 60.7%. The level of resistance to many antibiotics increased in 2015 and

2017, especially the resistance to carbapenem antibiotics in 2017 was 44.6%, approximately 2 times higher than the remaining years. The trends of increasing resistance to meropenem, imipenem and cefepim antibiotics were statistically significant ($p < 0.05$). There was a notable reduction in resistance to ertapenem, fosfomycin, amikacin, amoxicillin/calvulanic acid (Figure 6).

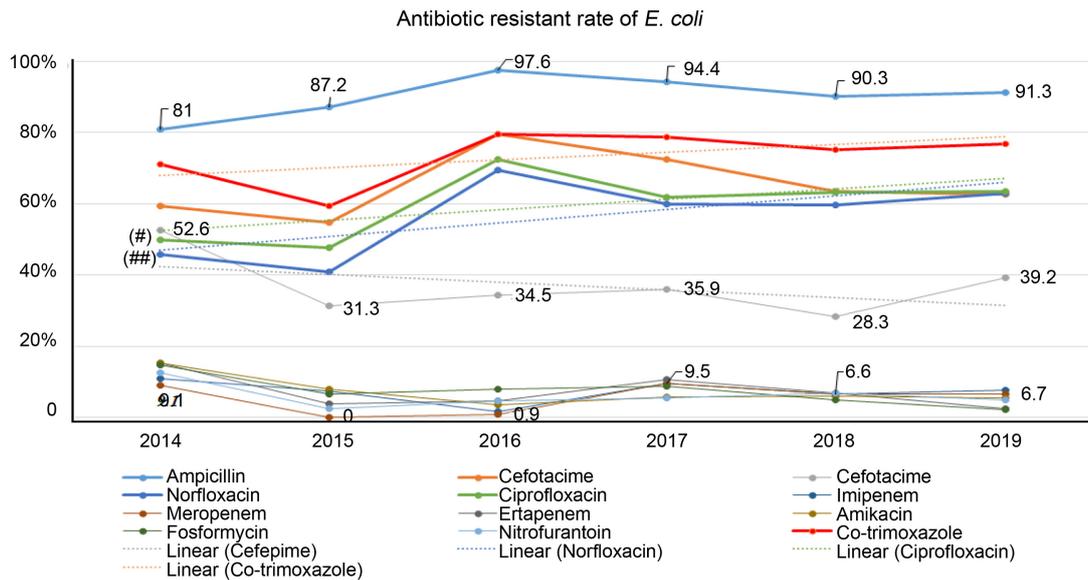


Figure 5. The resistant level of *E. coli* over time. # $p < 0.05$, a statistically significant result in Norfloxacin resistance during 2014-2019. ## $p < 0.05$, a statistically significant result in Ciprofloxacin resistance during 2014-2019.

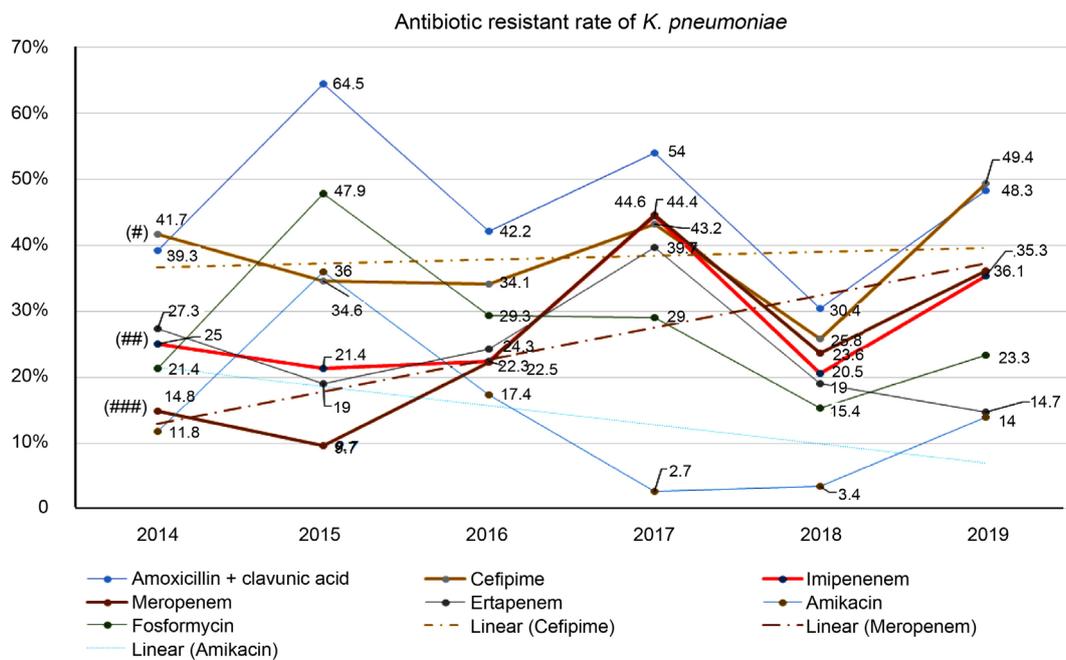


Figure 6. The resistant level of *K. pneumoniae* over time. # $p < 0.05$, a statistically significant result in Cefipime resistance during 2014-2019. ## $p < 0.05$, a statistically significant result in Imipenem resistance during 2014-2019. ### $p < 0.05$, a statistically significant result in Meropenem resistance during 2014-2019.

4. Discussion

In the short time the antibiotic was discovered and put into treating infections, the bacteria was able to resist them. It was found that the more antibiotics used, the faster they became resistant. In the race between finding a new antibiotic and the speed of increasing antibiotic resistance of bacteria, it seemed that bacteria always took the initiative. The natural selection pressure and the struggle for survival among bacteria have helped them an antibiotic resistant ability. Moreover, the combination of antibiotics in treatment led to multidrug resistance of bacteria [12].

During the study, we found that the percentage of more than 3-antibiotic group resistant bacteria was higher than the rate of less than 3-antibiotic group resistant bacteria in all four bacterial species. In partially, the rate of multi-resistant bacteria has increased over time. It led to a difficulty in selecting antibiotics for infection treatment in our hospital. Comparing with Japanese data in the 2018 annual monitoring report, the proportion of antibiotic resistant bacteria in 103 military hospital of Vietnam was much higher than the Japanese hospital (Table 1) [13].

The research and classify MDR, XDR and PDR strains were followed to the standards of the European Center for Disease Control and Prevention (ECDC) [12]. In this study, it was really worried that in all four common gram-negative bacilli, the rates of multi-drug resistant bacteria were more than 50% and this percentage had been increasing during the study period. For example, the multi-drug resistant rate of *A. baumannii* was 79.57% in 2017 and this figure was 86.8% in 2019. Moreover, MDR bacteria (non XDR or pPDR - resisted to more

Table 1. Comparison of antibiotic resistant bacteria strains between 103 military hospital and Japanese.

| | The 103 military hospital (n = 2209) | Japan (n = 2,891,652) | <i>P</i> |
|---|---|--------------------------|----------|
| Multidrug-resistant <i>P. aeruginosa</i> | 242 (11.0%) | 1082 (0.04%) | <0.05 |
| Multidrug-resistant <i>Acinetobacter</i> spp. | 257 (11.6%) | 99 (0.00%) | <0.05 |
| Carbapenem-resistant <i>Enterobacteriaceae</i> | 189 (8.6%) | 9304 (0.32%) | <0.05 |
| Carbapenem-resistant <i>P. aeruginosa</i> | 199 (9.0%) | 21202 (0.73%) | <0.05 |
| 3 rd Generation Cephalosporin-resistant <i>E. coli</i> | 342 (15.5%) | 92653 (3.2%) | <0.05 |
| 3 rd Generation Cephalosporin-resistant <i>K. pneumonia</i> | 136 (6.2%) | 14858 (0.51%) | <0.05 |
| Fluoroquinolone-resistant <i>E. coli</i> | 321 (14.5%) | 133170 (4.61%) | <0.05 |

than 3 antibiotic groups) have been replaced by XDR (only sensitive with one or two antibiotic groups) or possible PDR (may be resist all antibiotics) strains. In 2014, among the multidrug resistant *P. aeruginosa* strains, the XDR strain accounted for 67.86%, in 2019 this number increased to 90.32% (Figure 2). A multicenter study in Mexico that analyzed and assessed microbiological data from 47 units over 6 months showed that *K. pneumoniae* MDR (22.6%), *E. coli* (19.4%), *Enterobacter* spp (11.9%) and *P. aeruginosa* (8.8%). *A. baumannii* had the highest rate of XDR (8.8%) and no PDR strain has been detected [14] [15]. The announcement of an emergency resuscitation department in Tertiary India about the percentage of multidrug resistant *P. aeruginosa* strains, the MDR strains was 50%, XDR strains was 2.3% and no PDR strains found [15]. A survey of resistant bacteria trends in Mexico from 2005 to 2012 was also showed that *A. baumannii* increased MDR rate from 25.6% to 49.7% [6]. Although PDR strain had not found in the time of the study, the antibiotic resistant level of bacteria in this study was much higher than several studies in the world. This problem has warned a risk of transmission of multidrug-resistant strains, so we have to control closely hospital infections.

It can be said that *A. baumannii* was one of the most mentioned bacteria in the last 15 - 20 years, because it was the most serious nosocomial infection agent and resisted many different kinds of antibiotics. Therefore, WHO has been prioritized in research new antibiotics [2]. The results showed that the antibiotic resistant rates of *A. baumannii* were large with all most antibiotic groups such as cephalosporins, aminoglycosides, and fluoroquinolones. Even with carbapenem, which is a strong antibiotic, *A. baumannii* was not only resistant more than 50% but also increased over time. However, *A. baumannii* resisted amikacin and co-trimoxazole with low rates and decreased slowly every year. It is a good sign in selection of antibiotics for treatment *A. baumannii* (Figure 3). Other studies were shown the similar results in study *A. baumannii* from different areas [4] [5] [14] [16] [17] [18]. However, in a report from 2013 to 2017 of Malaysia were showed a difference. For example, *A. baumannii* was lower resistant imipenem, meropenem, and ceftazidime than our results with ranging from 55% - 62%, and it also resisted amikacin with the higher rates from 42% to 50% [19]. In another research from Mexico between 2005 and 2012 showed that the resistant percentage of *A. baumannii* to meropenem was from 17.7% to 33.0%, was not as high as the figure in this research [6]. In significant, in different time and regions the antibiotic resistant level of *A. baumannii* was different. Therefore, the monitoring of drug resistance should be conducted regularly and continuously.

Similar to *A. baumannii*, *P. aeruginosa* had a high level of resistance and increasing tendency with many antibiotics over time. This is a challenge in treating these strains in the near future. Here results were quite the same as the results from other studies [4] [20]. According to this research, *E. coli* were generally resistant rate lower than that of *A. baumannii* and *P. aeruginosa*. The rate of resistance increased over time such as cefotaxim, ciprofloxacin, and co-trimoxazole.

However, other antibiotics as amoxicillin/clavunanic acid, cefepime, fosformycin, nitrofurantoin, and amikacin decreased slowly the rate of resistance. Fortunately, the amikacin, fosformycin and carbapenem antibiotics are also good choices for clinicians because of high sensitivity. Nguyen Vinh Nghi in Ninh Thuan General Hospital of Vietnam was reported *E. coli* was resistant cephalosporin with the rate of 48.2% to 75%, and quinolone 72.7% and 73.2% higher than our research results, because these antibiotics have been used popularly in recent years. However, the resistant rates of *E. coli* with carbapenem and aminoglycoside were 0.7% and 1.4%, lower than this study [21]. The reason for this difference was probably these two antibiotics from reserve group. Provincial hospitals rarely used them for treatment meanwhile they were applied in most of the central hospitals. In South Korea, the 2017 surveillance report showed that the levels of antibiotic resistance of *E. coli* isolated from blood samples were 65.3%, 32.4%, 11.8% and 20.3% to ampicillin, cefotaxim, ceftazidim and cefepime respectively, whereas, the rates of carbapenem and amikacin were very low, accounting for 0.2% and 0.8% [22]. In other countries such as Japan and Malaysia, the rates of carbapenem and amikacin resistance of *E. coli* remained lower than 2% over the years and lower than 30% with cephalosporin [13] [19]. In some countries such as Russia, Serbia, Switzerland, Montenegro, and Ukraine, the rates of carbapenem resistance of *E. coli* isolated from blood and cerebrospinal fluid samples were lower than 2% [23]. *E. coli* is an agent of the intestinal microflora and it can cause opportunistic diseases, especially nosocomial infections. Here, the drug resistant levels of *E. coli* were bigger than other studies from different countries, probably Vietnam is a developing country, and environmental sanitation is not really good.

Like *E. coli*, *A. baumannii*, and *P. aeruginosa*, *K. pneumoniae* also plays an important role in nosocomial infections. According to this research, *K. pneumoniae* had high levels of resistance with many different antibiotics and higher resistant level than other studies [19] [20] [21] [22] [24] [25]. The carbapenem resistant rate increased significantly from 10% to 45%. Fortunately, *K. pneumoniae* still tended to significantly reduce the resistance with some antibiotics like ertapenem, fosfomycin, amikacin, and amoxicillin/calvulanic acid. It can be said that selecting antibiotics for treatment of *A. baumannii* and *P. aeruginosa* strains are more difficult than choosing drug in treatment for *E. coli* and *K. pneumoniae* strains.

5. Conclusions

Although the study was conducted in only a hospital, it is a military central hospital in Vietnam, where treated for a lot of serious patients with diverse diseases from different locals in the North of Vietnam. Thus, it can reflect the disease situation in a big area. The regular update on the level of antibiotic resistance of Gram-negative bacteria in 103 Military Hospital is always necessary. It provides useful information for clinical physicians in selecting antibiotics for treatment,

contributes in improving the quality of treatment and limiting the spread of antibiotic-resistant bacteria strains. In summary, our results indicated a trend of increasing resistance over time and a high resistant rate with many antibiotics of Gram-negative strains at a military hospital in Vietnam. In general, The XDR or pPDR strains had accounted a great proportion. It was a major challenge in the selection of antibiotics for treatment. However, results also showed that each bacterium was susceptible with some antibiotics. Depending on these results, clinicians have the best decision in choosing antibiotics for treatment as well as establishing appropriate strategies in using antibiotic. It is essential to regularly monitor of bacterial resistant levels, and should pay attention to more in-depth studies related to drug resistance such as resistance mechanisms, transmission routes of resistance strains and intensify control of nosocomial infections to limit the spread of multi-resistant strains.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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