Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Temporal association between antibiotic use and resistance in Gram-negative bacteria

Associação temporal entre o uso de antibióticos e resistência em bactérias gram-negativas

X. Wu[a#], G. Zhong[b#], H. Wang[c*] and J. Zhu[d]

[a]Central South University, Affiliated Haikou Hospital of Xiangya Medical College, Department of Clinical Laboratory, Haikou, Hainan, China
[b]Haikou Hospital of the Maternal and Child Health, Department of Pharmacy, Haikou, Hainan, China
[c]Hainan Provincial People's Hospital (Hainan Hospital Affiliated to Hainan Medical College), Department of Health Management Centre, Haikou, Hainan, China
[d]The Third Xiangya Hospital of Central South University, Department of Laboratory Medicine, Changsha, Hunan, China

Abstract

The β-lactam/lactamase inhibitors (BLBLIs) combination drugs are considered an effective alternative to carbapenems. However, there is a growing concern that the increased use of BLBLIs may lead to increased resistance. This study determined the temporal association between the consumption of BLBLI and the antimicrobial resistance in Gram-negative bacteria. In this retrospective study, electronic data on the Gram-negative bacterial isolates, including *A. baumannii, P. aeruginosa, E. coli,* and *K. pneumoniae* from in-patients and susceptibility testing results were retrieved from the medical records of the clinical laboratory. A linear regression and cross-correlation analysis were performed on the acquired data. Increasing trends ($p<0.05$) in the consumption of BIBLI and carbapenem with a median use of 27.68 and 34.46 DDD/1000 PD per quarter were observed, respectively. A decreased trend ($p=0.023$) in the consumption of fluoroquinolones with a median use of 29.13 DDD/1000 PD per quarter was observed. The resistance rate of *K. pneumoniae* was synchronized with the BIBLI and carbapenem consumptions with a correlation coefficient of 0.893 ($p=0.012$) and 0.951 ($p=0.016$), respectively. The cross-correlation analysis against the consumption of BIBLI and meropenem resistant *K. pneumoniae* was peaked at 0-quarter lag ($r=951$, $p=0.016$). There was an increasing trend in the consumption of BLBLI and carbapenems. The increasing trend in the rates of resistance to piperacillin/tazobactam, in line with the increasing consumption of BLBLI, suggests that BLBLI has to be used with caution and cannot be directly considered as a long-term alternative to carbapenems.

Keywords: β-lactam/lactamase inhibitors, temporal association of antibiotic resistance, Gram-negative bacteria.

Resumo

Os medicamentos combinados de β-lactâmicos / inibidores da lactamase (BLBLIs) são considerados uma alternativa eficaz aos carbapenêmicos. No entanto, existe uma preocupação crescente de que o aumento do uso de BLBLIs pode levar ao aumento da resistência. Este estudo determinou a associação temporal entre o consumo de BLBLI e a resistência antimicrobiana em bactérias gram-negativas. Neste estudo retrospectivo, os dados eletrônicos sobre as bactérias gram-negativas isoladas, incluindo *A. baumannii*, *P. aeruginosa*, *E. coli* e *K. pneumoniae* de pacientes internados e os resultados dos testes de suscetibilidade foram recuperados dos registros médicos do laboratório clínico. Uma regressão linear e análise de correlação cruzada foram realizadas nos dados adquiridos. Foram observadas tendências crescentes ($p < 0,05$) no consumo de BIBLI e carbapenem com uma mediana de uso de 27,68 e 34,46 DDD/1000 PD por trimestre, respectivamente. Foi observada uma tendência de diminuição ($p = 0,023$) no consumo de fluoroquinolonas com uma mediana de uso de 29,13 DDD/1000 PD por trimestre. A taxa de resistência de *K. pneumoniae* foi sincronizada com os consumos de BIBLI e carbapenem com coeficiente de correlação de 0,893 ($p = 0,012$) e 0,951 ($p = 0,016$), respectivamente. A análise de correlação cruzada contra o consumo de BIBLI e *K. pneumoniae* resistente ao meropenem atingiu o pico no intervalo de 0 quarto ($r = 951$, $p = 0,016$). Houve uma tendência de aumento no consumo de BLBLI e carbapenêmicos. A tendência crescente nas taxas de resistência a piperacilina/tazobactam, em linha com o consumo crescente de BLBLI, sugere que BLBLI deve ser usado com cautela e não pode ser considerado diretamente como alternativa de longo prazo aos carbapenêmicos.

Palavras-chave: inibidores β-lactâmicos/lactamase, associação temporal de resistência a antibióticos, bactérias gram-negativas.

*e-mail:18289543449@139.com
Received: June 9, 2020 – Accepted: January 1, 2021
#These two authors contributed to the work equally.

1. Introduction

Gram-negative bacteria, *Acinetobacter baumannii*, *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumoniae* are associated with nosocomial infections, including urinary tract infections, bloodstream infections, catheter-associated infections and ventilator-associated pneumonia (Peleg and Hooper, 2010). The increasing drug resistance towards various antibiotics leads to severe life-threatening conditions and poses a challenge in the treatment of Gram-negative bacterial infections. In addition, the emergence of multi-drug resistant (MDR) Gram-negative bacteria adds to the burden of treatment challenges and increased morbidity and mortality (Gurjar et al., 2013; Righi et al., 2017; Shah and Shah, 2015; Yang et al., 2021). By 2050, in the Asia-Pacific region, an estimated 4.73 million deaths will occur annually due to global antimicrobial-resistant and will lead to a comprehensive economic loss of up to $100 trillion (Shah and Shah, 2015; Teillant et al., 2015). Beta-lactam antibiotics were the drug of choice for the treatment of Gram-negative bacterial infections. However, the emergence of extended-spectrum β-lactamase (ESBL) producing microorganisms raised a global alarm. Subsequently, carbapenems were introduced and effectively used against ESBL producing and MDR Gram-negative bacteria (Livermore and Woodford, 2006; Mansy et al., 2020). Worldwide, MDR organisms, including *A. baumannii, P. aeruginosa*, ESBL- or carbapenemase-producing Enterobacteriaceae have been increasingly reported (Peleg and Hooper, 2010). Reports on carbapenem resistance have raised serious concern and indicated the limited therapeutic options available for the treatment (Lee et al., 2005). To overcome carbapenem resistance, β-lactam/lactamase inhibitors (BLBLIs) combination drugs such as piperacillin/tazobactam and ampicillin/sulbactam were introduced to increase the efficacy of β-lactam antibiotics (Drawz and Bonomo, 2010). However, there is a growing concern that the increased use of BLBLIs may lead to increased resistance.

Studies evaluating the association of antibiotic consumption and resistance are as old as the antibiotics. They are affected by several parameters, including the extent of prior antibiotic exposure, control group selection, measurements of resistance outcomes and inadequate adjustment for important confounding variables (Schechner et al., 2013). Thus, several indicators were used to measure antibiotic use; however, it is still debatable on which is the best. Three different metrics viz., (i) defined daily dose (DDD), (ii) days of therapy (DOT), (iii) length of therapy (LOT) are available for the measurement of antibiotic use. However, the World Health Organization (WHO) proposed DDD as the commonly used metric; it is expressed as DDDs per 100,000 population (for outpatient use) and DDDs per 1,000 patient-days (for in-patient use) (WHOCC, 2013). The DDD metrics method is easy to collect, no patient-level data is required, it allows standardized comparisons between drugs and between settings irrespective of route. The other two metrics require patient-level data to be collected (Schechner et al., 2013). Hence, in our study, we have adapted the DDD metrics to measure antibiotic use. There is a general agreement that the increased use of antibiotics will lead to increased resistance (Hsueh et al., 2005). The association between BLBLI consumption and antibiotic resistance Gram-negative bacteria is still unclear. Our study aimed to determine the association between the consumption of BLBLI, carbapenems, third-generation cephalosporin's, fluoroquinolones, and antibiotic resistance (representative antibiotics of each group) in Gram-negative bacteria, including *A. baumannii, E. coli, P. aeruginosa,* and *K. pneumoniae*. It also determined the temporal association between the consumption of BLBLI and the antimicrobial resistance in Gram-negative bacteria for better decision making on the appropriate use of antibiotics.

2. Methods

This retrospective study was conducted in The Third Xiangya Hospital and Changsha Central Hospital, China between January 2016 and December 2019. Prior to the study, ethics committee approval was obtained from the hospital ethics committee (No. 0023-15). Electronic data on the Gram-negative bacterial isolates, including *A. baumannii, E. coli, P. aeruginosa,* and *K. pneumoniae* from in-patients and susceptibility testing results were retrieved from the medical records of the clinical laboratory. Only the non-repetitive Gram-negative bacteria data were considered for this analysis. API 20E kits (Biomérieux, France) identified isolates. Antimicrobial susceptibility testing was done by Kirby-Bauer Disk Diffusion method according to guidelines of Clinical and Laboratory Standards Institute published in the respective years of testing. Antibiotic consumption rate was acquired for BIBLI, carbapenem, third-generation cephalosporin and fluoroquinolones class of drugs. Antibiotic consumption was acquired as per WHO 2012 anatomic therapeutic chemical (ATC) classification in DDD/1000 patient-days (PD) (WHOCC, 2019). Since the antibiotic resistance mechanism is similar among the same classes of antibiotics, the ATC classification is widely used to measure the relationship between antibiotic resistance and antibiotic exposure by bacterial isolates (Goossens, 2009). For this study, resistance data for one antibiotic representing each group was used for correlation analyses; piperacillin/tazobactam represented BIBLI, meropenem represented the carbapenems, ceftazidime represented the third-generation cephalosporin's and ciprofloxacin represented the fluoroquinolones.

A linear regression analysis and cross-correlation function analysis were performed. Linear regression analysis was performed to evaluate the trends of antibiotic consumption and antibiotic resistance. A p-value of <0.05 and the R square value of >0.3 was considered as statistically significant. Cross-correlation function analysis performed to assess the association between the quarterly isolation rate of antibiotic susceptibility and a quarterly number of antibiotic usage. The maximum positive correlation coefficient, the lag quarter and its P value were obtained from cross-correlation function analyses. Statistical analyses were performed using MINITAB Ver. 13 (PA, USA) statistical software.

3. Results

3.1. Antibiotic consumption

Increasing trends ($p<0.05$) in the consumption of BIBLI and carbapenem with a median use of 27.68 and

34.46 DDD/1000 PD per quarter were observed, respectively. A decreased trend (p=0.023) in the consumption of fluoroquinolones with a median use of 29.13 DDD/1000 PD per quarter was observed. The consumption of third-generation cephalosporin was stable over the study period (Figure 1).

3.2. Antibiotic resistance

The mean antibiotic resistance rates of *A. baumannii* against piperacillin/tazobactam, meropenem, ceftazidime and ciprofloxacin were respectively 55.5% (range 54.3%-56.5%), 6% (5.2%-6.3%), 38.2% (28.6%-49.1%) and 62.6% (57.1%-72.4%). The mean *E. coli* resistance rates against the same antibiotics were 31.4% (26.3%-36.2%), 6.3% (5.9%-7.1%), 38.7% (32.6%-42.3%) and 55.9% (48.6%-62.3%), respectively. The mean *P. aeruginosa* resistance rates against the same antibiotics were 49.1% (45.6%-52.3%), 5% (4.6%-5.4%), 42.4% (31.2%-50.3%) and 58.2% (49.6%-68.5%), respectively. The mean *K. pneumoniae* resistance rates against the same antibiotics were 36.2% (28.3%-42.3%), 9.5% (7.5%-10.6%), 28% (25.4%-31.5%) and 59.9% (56.4%-64.3%), respectively. Overall, irrespective of the microorganisms, ciprofloxacin resistance rates were highest ranging from 48.6%-72.4%; while meropenem resistance rates were lowest ranging from 4.6%-10.6%.

The resistance rate of *K. pneumoniae* showed an increased, steadily increased, and decreased trend towards piperacillin/tazobactam, meropenem and ceftazidime over the study period, respectively. The resistance rate of *A. baumannii* showed an increased and a marginal decrease trend towards ceftazidime and piperacillin/tazobactam, respectively. The resistance rate of *P. aeruginosa* showed an increased trend towards ceftazidime and piperacillin/tazobactam. The resistance rate of *E. coli* showed a decreased trend towards piperacillin/tazobactam while it maintained almost a stable trend against all the other antibiotics included in this study. Ciprofloxacin resistance was almost stable in all microorganisms included in this study (Figure 2).

3.3. Correlation and cross-correlation analysis

The resistance rate of *K. pneumoniae* was synchronized with the BIBLI and carbapenem consumptions with a correlation coefficient of 0.893 (p=0.012) and 0.951 (p=0.016), respectively. *E. coli* resistance rate showed a negative correlation with the consumption of BIBLI with a correlation coefficient of 0.428 (p=0.047), respectively. *E. coli* resistance remained stable against ceftazidime and did correlate well with the stable use of third-generation cephalosporin's (r=0.714, p=0.027). The resistance rate of *A. baumannii* was on an increasing trend against ceftazidime and did not correlate with the stable use of third-generation cephalosporin's (r=0.262, p=0.141). There was a weak correlation between the *P. aeruginosa* resistance and the consumption of piperacillin/tazobactam (r=0.305, p=0.045) and ceftazidime (r=0.301, p=0.039). The cross-correlation analysis against the consumption of BIBLI and meropenem resistant *K. pneumoniae* was peaked at 0-quarter lag (r=951, p=0.016) (Table 1).

4. Discussion

The overuse and misuse of antibiotics is the major cause of dissemination of antibiotic resistance (Zhang et al., 2019). There was an increasing trend (p<0.05) in the consumption of BIBLI with a median use of 27.68 and 34.46 DDD/1000 PD per quarter was observed in our study. Similar results were published in a study from Korea on *K.*

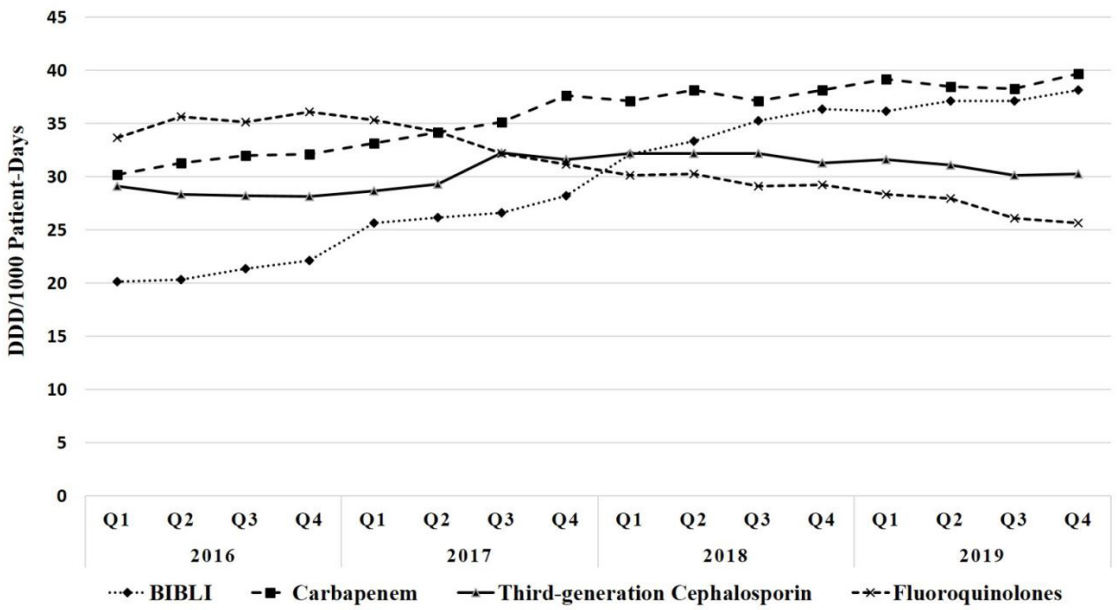

Figure 1. Antibiotic consumption trend.

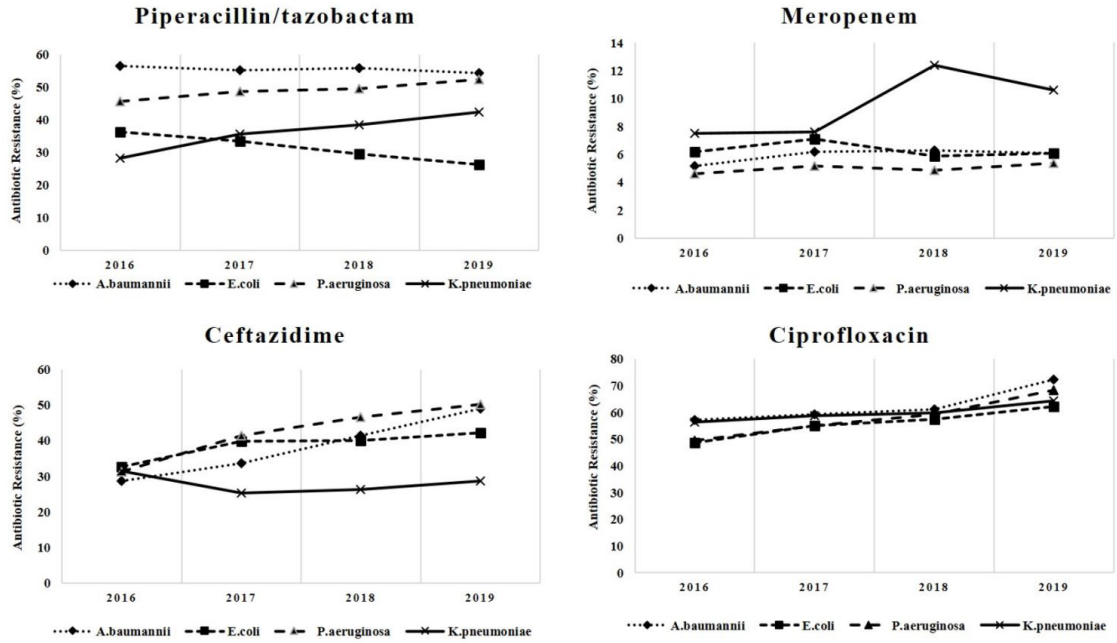

Figure 2. Antibiotic resistance trend among Gram negative bacteria.

Table 1. Cross-correlation analysis between the consumption of BLBLI and antibiotic resistance of Gram-negative bacteria.

Microorganisms	Piperacillin/ tazobactam	Meropenem	Ceftazidime	Ciprofloxacin
A. baumannii	r=0.285 p=0.237 Lag 0	r=0.301 p=0.347 Lag 1	r=0.262 p=0.141 Lag 0	r=0.361 p=0.136 Lag 0
E. coli	r=0.428* p=0.047 Lag 1	r=0.381 p=0.213 Lag 2	r=0.714 p=0.027 Lag 0	r=0.221 p=0.198 Lag 1
P. aeruginosa	r=0.305 p=0.045 Lag 0	r=0.251 p=0.416 Lag 0	r=0.301 p=0.039 Lag 1	r=0.298 p=0.452 Lag 0
K. pneumoniae	r=0.893 p=0.012 Lag 0	r=0.951 p=0.016 Lag 0	r=0.325 p=0.471 Lag 0	r=0.312 p=0.365 Lag 0

*Negative Correlation.

pneumoniae (Ryu et al., 2018). In addition, we reported that there was an increasing trend in the use of carbapenems, which is similar to that reported in China (Hao et al., 2020; Zhang et al., 2019). We reported a median increased trend ($p<0.05$) in the consumption of carbapenem with a median use of 34.46 DDD/1000 PD per quarter. A study result from China exactly corroborates our findings; the study reported an increasing trend of carbapenem consumption with an average of 32.68 DDDs/1,000 pd (Hao et al., 2020). According to a report from Europe, the average consumption of carbapenem was only 0.05 DDDs/1,000 pd, which is much lesser than that reported in our study (ECDC, 2016). China is considered as the largest antibiotic consumer in the world (Heddini et al., 2009), explains the largest BLBLI and carbapenem consumption compared to European data. Our hospital is a tertiary care centre; handling a large number of critical care patients with multiple diseases could also be another reason for the highest consumption of both BLBLI and carbapenem drugs.

The increasing rate of ESBL producing organisms, including *A. baumannii, P. aeruginosa*, *K. pneumoniae* has raised serious concern and limits the clinical therapeutic options (Lee et al., 2005; Paterson and Bonomo, 2005; Peleg and Hooper, 2010). Although carbapenems are considered effective against ESBL producing Gram-negative bacteria, the overconsumption of carbapenems led to ineffective treatment. The BLBLI is considered as an effective alternative to carbapenems (Tamma and Rodriguez-Bano, 2017). However, the increased use of these drugs led to increased resistance, which undermines the efficacy of these drugs

(Tamma and Rodriguez-Bano, 2017). Establishing the temporal association between the use of antibiotics and the resistance rates will help in developing appropriate protocol for BLBLI. Several studies have focused on the temporal association of antibiotic use and antibiotic resistance (Goossens, 2009; Hao et al., 2020; Mansy et al., 2020; Ryu et al., 2018).

In our study, there was a positive temporal correlation observed between BLBLI utilization and piperacillin/tazobactam resistance in *K. pneumoniae*. Our result is in complete agreement with previous studies (Mansy et al., 2020; Ryu et al., 2018). Similarly, our findings are consistent with the results of another study, which reported a significant correlation between the use of piperacillin/tazobactam and its resistance in *Enterobacteriaceae* (Lai et al., 2011). Another study reported that *Klebsiella* sp. showed a significant positive correlation with BLBLI, cephalosporin, and fluoroquinolones, while in our study, there was a positive correlation with only BLBLI and a negative correlation with third-generation cephalosporin use (Wang et al., 2004). Similar to piperacillin/tazobactam resistance, in our study, there was a positive temporal correlation between the carbapenem utilization and meropenem resistance in *K. pneumoniae*. However, there was no significant association between the use of fluoroquinolones and ciprofloxacin-resistant *K. pneumoniae*. In our study, there was a significant correlation between the use of third-generation cephalosporin and ceftazidime resistant *E. coli*. The consumption rate of third-generation cephalosporin was stable; consistent with its consumption, the ceftazidime resistance in *E. coli* was also found to establish a stable trend. While ceftazidime resistance in *K. pneumoniae* showed a negative correlation to the use of third-generation cephalosporin. The low level of fluctuation and low magnitude of the utilization of fluoroquinolones could be the possible reason for our results (Peterson, 2005). In contrast, a previous study reported a positive correlation between ceftazidime resistance in *K. pneumoniae* and the use of third-generation cephalosporin (Mansy et al., 2020). The fluoroquinolone use was in a declining trend, although not significantly correlated the ciprofloxacin resistance in *A. baumannii, E. coli, P. aeruginosa* and *K. pneumoniae* were on a slightly increasing trend. These could possibly be due to selection pressure of antibiotic usage on resistant isolates (Mansy et al., 2020; Ryu et al., 2018). The cross-correlation analysis against the consumption of BIBLI and meropenem resistant *K. pneumoniae* was peaked at a 0-quarter lag. The first study, which determined the temporal association of BIBLI consumption resistance to *K. pneumoniae*, reported a significant temporal correlation between BLBLI use and piperacillin/tazobactam resistance in *K. pneumoniae* (Ryu et al., 2018). The strong correlation on the resistant Gram-negative bacteria with the increasing trend in the consumption of BLBLI suggests that BLBLI should be used with caution and cannot be considered as an ideal long-term alternate to carbapenems. As the consumption of BLBLI is on the rise with an increasing trend in the antibiotic resistance, we suggest that BLBLI may be used as along with carbapenems or as carbapenem sparing-option (Peterson, 2005).

The following are the limitations of our study: first, the study was not conducted at the individual level; hence should be considered as an ecological level and may not reflect the biological association at the individual level. Second, this is a bi-centric study; hence the result could probably be based on the local patient population and infection control measures adopted in our hospitals, and one should be cautious in applying these results for the general population of China. Thus, a larger multicentre study involving larger patient population and wide drug classes is required to evaluate the antibiotic stewardship program to balance the use and resistance of Gram-negative microorganisms against different classes of antibiotics.

5. Conclusion

There was an increasing trend in the consumption of BLBLI and carbapenems. The increasing trend in the rates of resistance to piperacillin/tazobactam, in line with the increasing consumption of BLBLI, suggests that BLBLI should be used with caution and cannot be directly considered as a long-term alternative to carbapenems.

References

DRAWZ, S.M. and BONOMO, R.A., 2010. Three decades of beta-lactamase inhibitors. *Clinical Microbiology Reviews*, vol. 23, no. 1, pp. 160-201. http://dx.doi.org/10.1128/CMR.00037-09. PMid:20065329.

EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL – ECDC, 2016 [viewed 21 March 2020]. *Antimicrobial consumption — Annual Epidemiological Report for 2016* [online]. Available from: https://ecdc.europa.eu/sites/portal/files/documents/AER_for_2016-AMC.pdf

GOOSSENS, H., 2009. Antibiotic consumption and link to resistance. *Clinical Microbiology and Infection*, vol. 15, suppl. 3, pp. 12-15. http://dx.doi.org/10.1111/j.1469-0691.2009.02725.x. PMid:19366364.

GURJAR, M., SAIGAL, S., BARONIA, A.K., RAO, B.P., AZIM, A., PODDAR, B. and SINGH, R.K., 2013. Carbapenem-resistant Acinetobacter ventilator-associated pneumonia: clinical characteristics and outcome. *Indian Journal of Critical Care Medicine : Peer-Reviewed, Official Publication of Indian Society of Critical Care Medicine*, vol. 17, no. 3, pp. 129-134. http://dx.doi.org/10.4103/0972-5229.117036. PMid:24082608.

HAO, Y., CHEN, S., CHANG, H., YAN, X., ZHOU, W., CAO, X., HUANG, R., ZHANG, Z., ZHANG, H., JIA, B., WU, W., XIONG, Y., CHEN, Y. and WU, C., 2020. Temporal association between Carbapenems usage and antimicrobial resistance in gram-negative bacteria at a tertiary hospital in Nanjing, China. *Diagnostic Microbiology and Infectious Disease*, vol. 98, no. 2, pp. 115083. http://dx.doi.org/10.1016/j.diagmicrobio.2020.115083. PMid:32650282.

HEDDINI, A., CARS, O., QIANG, S. and TOMSON, G., 2009. Antibiotic resistance in China--a major future challenge. *Lancet*, vol. 373, no. 9657, pp. 30. http://dx.doi.org/10.1016/S0140-6736(08)61956-X. PMid:19121722.

HSUEH, P.R., CHEN, W.H. and LUH, K.T., 2005. Relationships between antimicrobial use and antimicrobial resistance in Gram-negative bacteria causing nosocomial infections from 1991-2003 at a university hospital in Taiwan. *International*

Journal of Antimicrobial Agents, vol. 26, no. 6, pp. 463-472. http://dx.doi.org/10.1016/j.ijantimicag.2005.08.016. PMid:16280243.

LAI, C.C., WANG, C.Y., CHU, C.C., TAN, C.K., LU, C.L., LEE, Y.C., HUANG, Y.C., LEE, P.I. and HSUEH, P.R., 2011. Correlation between antibiotic consumption and resistance of Gram-negative bacteria causing healthcare-associated infections at a university hospital in Taiwan from 2000 to 2009. *The Journal of Antimicrobial Chemotherapy*, vol. 66, no. 6, pp. 1374-1382. http://dx.doi.org/10.1093/jac/dkr103. PMid:21436153.

LEE, C.M., LIM, H.K., LIU, C.P. and TSENG, H.K., 2005. Treatment of pan-drug resistant *Acinetobacter baumannii. Scandinavian Journal of Infectious Diseases*, vol. 37, no. 3, pp. 195-199. http://dx.doi.org/10.1080/00365540510026869. PMid:15849052.

LIVERMORE, D.M. and WOODFORD, N., 2006. The beta-lactamase threat in Enterobacteriaceae, *Pseudomonas* and *Acinetobacter*. *Trends in Microbiology*, vol. 14, no. 9, pp. 413-420. http://dx.doi.org/10.1016/j.tim.2006.07.008. PMid:16876996.

MANSY, W., MUZAHEED and RATHOD, S., 2020. Temporal association between antibiotic use and resistance in Gram-negative bacteria. *Archives of Pharmacy Practice (Mumbai)*, vol. 11, no. 2, pp. 13-18.

PATERSON, D.L. and BONOMO, R.A., 2005. Extended-spectrum beta-lactamases: a clinical update. *Clinical Microbiology Reviews*, vol. 18, no. 4, pp. 657-686. http://dx.doi.org/10.1128/CMR.18.4.657-686.2005. PMid:16223952.

PELEG, A.Y. and HOOPER, D.C., 2010. Hospital-acquired infections due to gram-negative bacteria. *The New England Journal of Medicine*, vol. 362, no. 19, pp. 1804-1813. http://dx.doi.org/10.1056/NEJMra0904124. PMid:20463340.

PETERSON, L. R., 2005. Squeezing the antibiotic balloon: the impact of antimicrobial classes on emerging resistance. *Clinical Microbiology and Infection: the Official Publication of the European Society of Clinical Microbiology and Infectious Diseases*, vol. 11, suppl. 5, pp. 4-16. https://doi.org/10.1111/j.1469-0691.2005.01238.x. PMID: 16138814.

RIGHI, E., PERI, A.M., HARRIS, P.N., WAILAN, A.M., LIBORIO, M., LANE, S.W. and PATERSON, D.L., 2017. Global prevalence of carbapenem resistance in neutropenic patients and association with mortality and carbapenem use: systematic review and meta-analysis. *The Journal of Antimicrobial Chemotherapy*, vol. 72, no. 3, pp. 668-677. PMid:27999023.

RYU, S., KLEIN, E.Y. and CHUN, B.C., 2018. Temporal association between antibiotic use and resistance in *Klebsiella pneumoniae* at a tertiary care hospital. *Antimicrobial Resistance and Infection Control*, vol. 7, no. 1, pp. 83. http://dx.doi.org/10.1186/s13756-018-0373-6. PMid:30026941.

SCHECHNER, V., TEMKIN, E., HARBARTH, S., CARMELI, Y. and SCHWABER, M.J., 2013. Epidemiological interpretation of studies examining the effect of antibiotic usage on resistance. *Clinical Microbiology Reviews*, vol. 26, no. 2, pp. 289-307. http://dx.doi.org/10.1128/CMR.00001-13. PMid:23554418.

SHAH, P.G. and SHAH, S.R., 2015. Treatment and Outcome of Carbapenem-Resistant Gram-Negative Bacilli Blood-Stream Infections in a Tertiary Care Hospital. *The Journal of the Association of Physicians of India*, vol. 63, no. 7, pp. 14-18. PMid:26731822.

TAMMA, P.D. and RODRIGUEZ-BANO, J., 2017. The Use of Noncarbapenem β-Lactams for the Treatment of Extended-Spectrum β-Lactamase Infections. *Clinical Infectious Diseases*, vol. 64, no. 7, pp. 972-980. http://dx.doi.org/10.1093/cid/cix034. PMid:28362938.

TEILLANT, A., GANDRA, S., BARTER, D., MORGAN, D.J. and LAXMINARAYAN, R., 2015. Potential burden of antibiotic resistance on surgery and cancer chemotherapy antibiotic prophylaxis in the USA: a literature review and modelling study. *The Lancet. Infectious Diseases*, vol. 15, no. 12, pp. 1429-1437. http://dx.doi.org/10.1016/S1473-3099(15)00270-4. PMid:26482597.

WANG, M., SAHM, D.F., JACOBY, G.A. and HOOPER, D.C., 2004. Emerging plasmid-mediated quinolone resistance associated with the qnr gene in *Klebsiella pneumoniae* clinical isolates in the United States. *Antimicrobial Agents and Chemotherapy*, vol. 48, no. 4, pp. 1295-1299. http://dx.doi.org/10.1128/AAC.48.4.1295-1299.2004. PMid:15047532.

WHO COLLABORATING CENTRE FOR DRUG STATISTICS METHODOLOGY – WHOCC, 2013 [viewed 31 March 2020]. *ATC/DDD index* [online]. Available from: http://www.whocc.no/atc_ddd_index/

WHO COLLABORATING CENTRE FOR DRUG STATISTICS METHODOLOGY – WHOCC, 2019. *Guidelines for ATC classification and DDD assignment 2020*. Oslo, Norway: WHOCC, pp. 1-247.

YANG, X., LAI, Y., LI, C., YANG, J., JIA, M. and SHENG, J., 2021. Molecular epidemiology of Pseudomonas aeruginosa isolated from lower respiratory tract of ICU patients. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 81, no. 2, pp. 351-360. http://dx.doi.org/10.1590/1519-6984.226309. PMid:32491054.

ZHANG, D., HU, S., SUN, J., ZHANG, L., DONG, H., FENG, W., LEI, J. and DONG, Y., 2019. Antibiotic consumption versus the prevalence of carbapenem-resistant Gram-negative bacteria at a tertiary hospital in China from 2011 to 2017. *Journal of Infection and Public Health*, vol. 12, no. 2, pp. 195-199. http://dx.doi.org/10.1016/j.jiph.2018.10.003. PMid:30385238.