Study of Hospital Acquired hypervirulent *Klebsiella pneumoniae* from Pediatric patients and its Relation to Carbapenem and Extended-Spectrum beta-lactamase Resistance.

1Mohamed Anies Rizk, 2Omnia Ahmed Mohamed Salem, 1Maysaa El Sayed Zaki, 3Mona Foda Salama, 4Mai Esam Ahmed

1 Clinical Pathology Department, Mansoura Faculty of Medicine, Mansoura University, Egypt

2Pediatric Department- Mansoura Faculty of Medicine, Mansoura University, Egypt

3 Medical Microbiology and Immunology Department, Mansoura Faculty of Medicine, Mansoura University, Egypt

4Clinical Pathology Department- Beni Suef Faculty of Medicine, Beni Suef University, Egypt

Abstract

Background: *Klebsiella pneumoniae* (*K. pneumoniae*) is a microorganism associated with hospital acquired infections worldwide. There is hypervirulent (hvKp) variant of this microorganism with enhanced virulent characters that may be associated with antibiotics resistance.

Aim: The aim of the present study was to detect the presence of hypervirulent *K. pneumoniae* among clinical isolates from pediatric patients with hospital acquired infections by Multiplex polymerase chain reaction (PCR). In addition, to study the association of this variety with carbapenem and Extended-Spectrum beta-lactamase (ESBL) antibiotics resistance.

Method: The study was a retrograde cross- sectional study that included 178 isolates of *K. pneuminae* from pediatric patients with hospital acquired infections. The isolates were subjected to antibiotics sensitivity test and detection of carbapenemase and ESBL activity study. Identification of hvKp variety was performed by multiplex PCR.

Results: Multiplex-PCR study for hvKp detected that 52 (29.2%) clinical isolates of *Klebsiella penumoniae* were positive. The hvKp was significantly associated with carbapenemase phenotype (P=0.001, OR 32.47, 95%CI 10.88-96.89) and with ESBL phenotype (P=0.001, OR 5.04, 95%CI 2.4-10.4). The association of hvKp was insignificant with MDR in isolated *K. pneumoniae* (P=0.52). The comparison between hvKp positive and negative types of *K. pneumonia* revealed significant increase in the resistance to carbapenem antibiotics imipenem and meropenem among hvKp positive

types (P=0.001 for each). The hvKp negative type of *K. pneumoniae* was significantly associated with high resistance to ceftazidime and tetracycline (P=0.001 for each).

Conclusion: The present study highlights the emergence of hvKp a variant of *K. pneumoniae* in children with hospital acquired infections. This variant is associated with antibiotics resistance to β-lactam antibiotics and carbapenem antibiotics. This finding is a warning finding which indicates changes in the spread of virulent with the need for implementation of strict antibiotics use and may need the use of new therapeutic agents.

Keywords: *K. pneumoniae*, hypervirulent, ESBL, carbapenemase, MDR, children.

Introduction

Klebsiella pneumoniae (*K. pneumoniae*) is a Gram-negative bacillus and one of the opportunistic pathogens *K. pneumoniae* is responsible for varieties of hospital acquired infections such as urinary tract infections, pneumonia and blood stream infections (1). It represents one pathogen known as ESKAPE group associated with antibiotics resistance. This acronym includes six bacterial pathogens including *Enterococcus faecium*, *Staphylococcus aureus*, *K. pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. *K. pneumoniae* organism is associated with the presence of extended-spectrum beta-lactamases (ESBLs) and carbapenem resistance (2). It has been listed by the World Health Organization (WHO) as a virulent organism with the need for the development of new antibiotics (3).

The pathogenicity of *K. pneumoniae* is also attributed to other virulence factors besides antibiotics resistance such as its capsule, lipopolysaccharide, types 1 and 3 fimbriae, siderophores, and allantoin metabolism (4); these characteristics are controlled by large virulence plasmids and integrated chromosomal elements. There are reports about the emergence of hypervirulence (hvKp) a variant of *K. pneumoniae* with high morbidity and mortality rates (5, 6). There is a global concern about the emergence of carbapenem resistance associated with hykp *K. pneumoniae* (7, 8). There are studies about the hykp variant in adults with limited studies about the association of this variety in hospital acquired infections in children (9).

There are various genetic markers that can be used for identification of hykp like prmpA/A2 (capsule production regulator), iuc (aerobactin synthesis), and peg344 (a

metabolic transporter of unknown function) (10, 11). Phenotypic identification can be used by the hypermucoviscosity detections of the isolated colonies (12).

There are previous reports about the emergence of hyper virulence *Klebsiella peumoniae* in adults patients with hospital acquired infections in Egypt (13, 14), but studies are limited in children patients.

The aim of the present study was to detect the presence of hypervirulent *K. pneumoniae* among clinical isolates of *K. pneumoniae* isolated from pediatric patients with hospital acquired infections by Multiplex polymerase chain reaction (PCR). In addition, to study the association of this variety with carbapenem and ESBL antibiotics resistance.

Material and Method

The study was a retrograde cross- sectional study that included *K. pneuminae* isolated from pediatric patients with hospital acquired infections from January 2019 till October 2021 from Mansoura University Children hospital, Egypt during the surveillance of hospital acquired infections. The diagnosis of hospital acquired infections was performed according to the criteria of Center of Diseases control (15). The study was approved by Mansoura Faculty of Medicine Ethical committee (R.23.02.2015). The study was performed according to the declaration of Helsinki ethical guidelines.

Microbiological Identification of *K.pneumoniae*

The isolated K. pneumoniae from the clinical samples other than blood samples were cultured on MacConkey agar media (Oxoid, Germany) for 24 hours at 37°C and identified with Gram stain and biochemical reactions by the use of Vitek system. Blood samples from children was obtained under complete sterile conditions and inoculated on Bact/ alert blood culture system. When blood culture bottles were found positive, subculture was performed on MacConkey agar media (Oxoid, Germany). Molecular identification was performed by PCR by the use of of polygalacturonase (pehX) gene as described previously (16).

Antibiotics Discs Sensitivity

The antibiotics sensitivity of the isolated *K. pneumoniae* was determined by the discs diffusion method according to the clinical and laboratory standards institute guidelines (CLSI) (17) . The discs used were ceftazidime (30µg), cefepime (30µg),

cephotaxime (5µg), imipenem (10µg), meropenem (10µg), cefoxitin (10µg), gentamicin (10µg), tetracycline (10µg) and trimethoprim/sulfamethoxazole (25µg) (Oxoid-Thermofisher-UK) The results were interpreted according to CSLI (17) Multi drugs resistant (MDR) *K. pneumoniae* was identified when the isolate was ≥ resistant to classes of antibiotics.

Detection of ESBL Activity by Combined Disc Test (CDT)

K. pneumoniae resistant to to ceftazidime and/ or cefotaxime was further studied for the ESBL activity by the CDT as described by CLSI (17). The isolates were subcultured on Muller-Hinton agar (Oxoid-Germany) with placing discs of ceftazidime and cefotaxime (30µg each) at a 20-mm distance from two discs of ceftazidime/clavulanic acid (30//10µg) and cefotaxime/ (30/10µg), respectively. The increase in the inhibition zones around clavulinic acids containing disc was more than 5 mm, these isolates were ESBL producing strains.

Phenotypic Detection of Carabapenemase

K. pneumoniae isolates resistant to imipenem and// or meropenem were further studied to carbapenemase activity by the EDTA discs method as described by CLSI method (17). The isolated K. pneumoniae were subcultured on Muller- Hinton agar media and discs of imipenem and meropenem were applied with and without 20µl of 0.5M EDTA with incubation at 37C for 24 hours.. The result was reported positive if the difference in the inhibition zone between meropenem and/or imipenem discs with or without EDTA was ≥5 mm (15).

Multiplex PCR for Identification of hykp *K. pneumoniae*

DNA Extraction of K. pneumoniae

DNA of the isolated K. pneumoniae was extracted by the the silica-based membrane technology and spin column by the use of commercial kit Gene JET genomic DNA purification kit according to the manufacturer instructions(Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA was kept frozen at -80°C till further amplification.

Multiplex PCR of hykp *K. pneumoniae*

Ready to use amplification kit (Applied Biosystems-USA) was used for the amplification by the method described previously. The used primers for the multiplex PCR were in previous study (11). The amplification conditions were an initial denaturation step of 94°C for 10 min, followed by 35 cycles at 94°C for 30 s, 60°C

for 30 s, 72°C for 60 s, and a final extension step of 72°C for 5 min. Amplicons were visualized after running at 100 V for 1.5 h in 1.5% agarose gels (11).

Statistical Analysis

The data of the study was analyzed by the SPPS22. The numerical data was expressed as mean and standard deviation if parametric and as minimum and maximum and median if non parametric. The qualitative data was expressed as number and percentages and the comparison was performed by Chi- square test, P was considered significant if <0.05.

Results

The study included 178 children with hospital acquired infections due to Klebsiella pneumonia. They were 106(59.9%) males and 72 (40.4%) females with age from one month up to 14.0 years. The clinical sources of the isolated *Klebsiella. pneumoniae* were sepsis in 41.6%, urinary tract infections (24.7%), wound infections (19.1%) and pneumonia (14.6%). The common associated devices were urinary tract catheter (50%), followed by central venous catheter (29.8%), table 1.

Antibiotics resistance of the isolated K. pneumonia revealed high résistance to the third generation of cephalosporines, Cefotaxime(66.3%), **cefepime(49.4%),** ceftazidime (49.4), cephotaxime (44.4%) and fourth generation cephalosporine Cefepime (49.4%) and marked resistance for carabepnem, imipenem and meropenem (46.6% and 45.5% respectively). Moreover, high resistance was detected toward trimethoprim/sulfamethoxazole (60.7%), tetracycline (64.04%) and gentamicin (41.6%), data not shown.

The phenotypic study of ESBL and carbapenemase resistance of *K. pneumoniae* revealed high frequency of ESBL (48.3%) and high frequency of carbapenemase (51.7%). MDR was detected in 69.7% 0f the isolates, table 2

Multiplex-PCR study for hvKp detected that 52 (29.2%) clinical isolates of *Klebsiella penumoniae* were positive.

The comparison between hvKp positive and negative types of K. pneumonia revealed significant increase in the resistance to carbapenem antibiotics imipenem and meropenem among hvKp positive types (P=0.001 for each). The hvKp negative type of K. pneumonia was significantly associated with high resistance to ceftazidime and tetracycline (P=0.001 for each), table 3

Table (1): Basic demographic and clinical data of the studied children

Sex	
Male	106 59.6%
Female	72 40.4%
Age	
Minimum	1.0 month
Maximum	14.0 years
Median	
Type of primary infections	
Sepsis	74 41.6
Urinary tract infections	44 24.7
Wound infections	34 19.1
Pneumonia	26 14.6
Associated devices	
Central venous catheter	53 29.8
Urinary tract catheter	89 50

Table (2); ESBL, carabapenemase and MDR of clinical *K. pneumoniae*

	No. %
Extended Spectrum Beta lactamase resistance	86 48.3
Carbapenemase resistance	82 51.7
MDR	124 69.7%

Table (3): Comparison of antibiotics resistance between Antibiotics resistance of the hvKp Positive and hvKp negative *K.pneumoniae*

	hvKp Positive (n=52)	hvKp Negative (n=126)	P
Cefepime	21	55	0.7
Ceftazidime	39	49	0.001
Imipenem	48	35	0.001
Meropenem	47	34	0.001
Cefoxitin	31	63	0.2
Gentamicin	25	49	0.25
Trimethoprim/sulfa	33	75	0.72
Tetracycline	24	90	0.001
Cephotaxime	31	82	0.2

The hvKp was significantly associated with carbapenemase phenotype (P=0.001, OR 32.47, 95%CI 10.88-96.89) and also significantly associated with ESBL phenotype (P=0.001, OR 5.04, 95%CI 2.4-10.4). The association of hvKp was insignificant with MDR in isolated K. pneumoniae (P=0.52), table 4

Table (4): Comparison of hvKp genotypes positive and negative regarding ESBL, carbapeneamse and MDR

	hvKp Positive (n=52) No. %	hvKp Negative (n=126) No. %	P	OR	95%CI
ESBL	39 75	47 37.3	0.001	5.04	2.4-10.4
Carbapeneamse	48 92.3	34 26.9	0.001	32.47	10.88-96.89
MDR	38 73.1	85 67.5	0.52	1.26	0.61-2.6

Discussion

Hospital acquired infections is a global risk for hospitalized patients depending upon the immune state of the patients, severity of the underlying diseases and the presence of antibiotics resistant microrganisms in the hospital environment (18). Hospitalized children are vulnerable groups of patients susceptible to these infections as they have immature immune response beside frequent use of invasive devices when admitted to the hospitals (19, 20).

In the present study, the underlying sources of isolated *K. pneumoniae* were mainly from blood stream infections, followed by urinary tract infections, wounds infections and pneumonia. This finding was similar in previous studies with blood stream infections, urinary tract infections and pneumonia the most common hospital acquired infections in children(21-25). This can be attributed to the frequent use of urinary catheter and central venous catheter in hospitalized patients (26, 27).. Central venous catheter represents a vehicle for enterance of colonized flore from the patients skin, through the hands of the health care workers, via contaminated solutions and via connections of line leadings to infections (21, 28), The reduction of invasive devices associated infections requires optimal adherence to the infection control guidelines such as proper hand hygiene, application of maximum sterile barrier precaution when insertion is performed and adequate skin asepsis and dressings, reduce inappropriate use of invasive procedures, and remove catheter as soon as it is no longer needed (27).

Antibiotics resistance of *Klebsiella pneumoniae* isolated from patients with hospital acquired infections represent health care problem worldwide. In the present study, there was marked resistance to antibiotics resistance of the isolated *K. pneumoniae* revealed high résistance to the third generation of cephalosporines, and fourth generation cephalosporine and marked resistance for carabepnem imipenem and meropenem. This resistance was reported in previous, studies (29- 31). *K. pneumoniae* is known for its antibiotics resistance to β-lactam antibiotics including a variety of other vital therapeutic drugs (31). There is an evidence that this organism may residue in the hospital environment acting as a source for infections with the need for strict infection prevention and control practices. (31).

Hypervirulent *Klebsiella pneumoniae* was detected in the present study by multiplex PCR in 29.2% of isolated *K. pneumoniae*. The prevalence of this variant of *K. pneumoniae* varied among different studies from 3.7% 37.8% in different

geographical regions (32-36). The differences in the detections can be attributed to the difference of the used genetic biomarkers used to identify the hypervirulent variety whether it is least one genetic biomarker or five virulence genes to identify hgKp such in the present study by multiplex PCR. Also, the prevalence rates differ according to the age of the included patients whether adults or children (37).

Antimicrobial resistance rate was generally low among hvKp isolates, with 25.9% multi-drug resistance and only 11.1% carbapenems resistance, therefore, the prevalence of CR-hgKp was also much lower than adults (23.3%, 30/129 vs. 34.2%, 360/1052) (32). The observed spread of hvKp in children and emphasize the urgent need for further epidemiological studies of hvKp

In hypervirulent variant of *K. pneumoniae*, MDR was insignificantly this online with previous studies (38). However, there was statistically significant association between this variant and ESBL and carabapemenase production. The presence of this association can be explained by the acquisition of plasmids of the virulence genes with the plasmids coding for antibiotics resistance (37). This finding is a warning finding which points to continuous evolving under abuse of antibiotics leading to outbreaks of hospital acquired infections in hospitalized children.

Conclusion

The present study highlights the emergence of hypervirulence(hvKp) a variant of K. pneumoniae in children with hospital acquired infections. This variant is associated with antibiotics resistance to β-lactam antibiotics and carbapenem antibiotics. This finding is a warning finding which indicates changes in the spread of virulent with the need for implementation of strict antibiotics use and may need the use of new therapeutic agents.

Author contributions

Mohamed Anies Rizk shared in the laboratory study, the draft preparation of the article and data analysis of the study and revision of the draft of the article. Omnia Ahmed Mohamed Salem collected clinical data of the studied children, and wrote the article. Maysaa El Sayed Zaki shared in the laboratory study, the draft preparation of the article and data analysis of the study. Mona Foda Salama shared in the laboratory study draft preparation of the article.. Mai Esam Ahmed shared in the laboratory study and draft preparation of the article. All authors have read and approved the final manuscript

Funding

Self-funded.

Availability of data and materials

The data of the present study is available at

https://data.mendeley.com/drafts/bsszk2txmw

Declarations

There is no any competing of interests for any of the authors

Ethics approval and informed consent to participate

The Mansoura Faculty approved the study of the Medicine Ethical Committee (R.23.02.2015). The study was performed according to the declaration of Helsinki. Informed written consent was obtained from each participant.

Consent for publication

Not applicable.

Competing interests

There are no competing interests for any of the authors.

References

1-Ballén V, Gabasa Y, Ratia C, Ortega R, Tejero M and Soto S (2021) Antibiotic Resistance and Virulence Profiles of *Klebsiella pneumoniae* Strains Isolated From Different Clinical Sources. *Front. Cell. Infect. Microbiol.* 11:738223. doi: 10.3389/fcimb.2021.738223

2-Gorrie, C.L., Mirčeta, M., Wick, R.R. *et al.* Genomic dissection of *Klebsiella pneumoniae* infections in hospital patients reveals insights into an opportunistic pathogen. *Nat Commun* **13**, 3017 (2022). https://doi.org/10.1038/s41467-022-30717-6

3-World Health Organization (2017). Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics (WHO Press), 1–7.

12-Du Q, Pan F, Wang C, Yu F, Shi Y, Liu W, Li Z, He P, Han D and Zhang H (2022) Nosocomial dissemination of hypervirulent *Klebsiella pneumoniae* with high-risk clones among children in Shanghai. *Front. Cell. Infect. Microbiol.* 12:984180. doi: 10.3389/fcimb.2022.984180

4-Paczosa, M. K., and Mecsas, J. (2016). Klebsiella pneumoniae: Going on the offense with a strong defense. Microbiol. Mol. Biol. Rev. 80 (3), 629–661. doi: 10.1128/MMBR.00078-15

5-Wang, J. L., Chen, K. Y., Fang, C. T., Hsueh, P. R., Yang, P. C., and Chang, S. C. (2005). Changing bacteriology of adult community-acquired lung abscess in Taiwan: Klebsiella pneumoniae versus anaerobes. Clin. Infect. Dis. 40 (7), 915– 922. doi: 10.1086/428574

6-Prokesch, B. C., TeKippe, M., Kim, J., Raj, P., TeKippe, E. M., and Greenberg, D. E. (2016). Primary osteomyelitis caused by hypervirulent klebsiella pneumoniae. Lancet Infect. Dis. 16 (9), e190–e195. doi: 10.1016/S1473-3099(16)30021-4

7-Karlsson, M., Stanton, R. A., Ansari, U., McAllister, G., Chan, M. Y., Sula, E., et al. (2019). Identification of a carbapenemase-producing hypervirulent klebsiella pneumoniae isolate in the united states. Antimicrob. Agents Chemother. 63 (7), e00519-19. doi: 10.1128/AAC.00519-19

8-Wozniak, J. E., Band, V. I., Conley, A. B., Rishishwar, L., Burd, E. M., Satola, S. W., et al. (2019). A nationwide screen of carbapenem-resistant klebsiella pneumoniae reveals an isolate with enhanced virulence and clinically undetected colistin heteroresistance. Antimicrob. Agents Chemother. 63 (5), e00107-19. doi: 10.1128/AAC.00107-19

9-Li, Y., Dong, L., Gao, W., Zhen, J., Dong, F., and Yao, K. (2021). Hypervirulent klebsiella pneumoniae infections in pediatric populations in Beijing, (2017- 2019): Clinical characteristics, molecular epidemiology and antimicrobial susceptibility. Pediatr. Infect. Dis. J. 40 (12), 1059–1063. doi: 10.1097/ INF.0000000000003253

10-Lam, M. M. C., Wyres, K. L., Duchêne, S., Wick, R. R., Judd, L. M., Gan, Y. H., et al. (2018). Population genomics of hypervirulent klebsiella pneumoniae clonalgroup 23 reveals early emergence and rapid global dissemination. Nat. Commun. 9 (1), 2703. doi: 10.1038/s41467-018-05114-7

11- Russo, T. A., Olson, R., Fang, C. T., Stoesser, N., Miller, M., MacDonald, U., et al. (2018). Identification of biomarkers for differentiation of hypervirulent klebsiella pneumoniae from classical k. pneumoniae. J. Clin. Microbiol. 56 (9), e00776-18. doi: 10.1128/JCM.00776-18

13-El-Mahdy R, El-Kannishy G, Salama H. Hypervirulent Klebsiella pneumoniae as a hospital-acquired pathogen in the intensive care unit in Mansoura, Egypt. Germs. 2018 Sep 3;8(3):140-146. doi: 10.18683/germs.2018.1141. PMID: 30250833; PMCID: PMC6141227

14-Ahmed MAEE, Yang Y, Yang Y, Yan B, Chen G, Hassan RM, Zhong LL, Chen Y, Roberts AP, Wu Y, He R, Liang X, Qin M, Dai M, Zhang L, Li H, Yang F, Xu L, Tian GB. Emergence of Hypervirulent Carbapenem-Resistant Klebsiella pneumoniae Coharboring a *bla*$_{NDM-1}$-Carrying Virulent Plasmid and a *bla*$_{KPC-2}$-Carrying Plasmid in an Egyptian Hospital. mSphere. 2021 May 19;6(3):e00088-21. doi: 10.1128/mSphere.00088-21. PMID: 34011682; PMCID: PMC8265623

15-Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of healthcare–associated infection and criteria for specific types of infections in the acute care setting. Am J Infect Control. 2008;36(5):309–32.

16-Kovtunovych G, Lytvynenko T, Negrutska V, Lar O, Brisse S, Kozyrovska N. Identification of Klebsiella oxytoca using a specific PCR assay targeting the polygalacturonase pehX gene. Res Microbiol. 2003 Oct;154(8):587-92. doi: 10.1016/S0923-2508(03)00148-7.

17-Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 28th editions. CLSI supplement M100-S28. Wayne: CLSI, 2018.

18- Monegro AF, Muppidi V, Regunath H. Hospital Acquired Infections. [Updated 2022 Aug 22]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441857/

19- de Mallo MJG, de Albuquerque MFPM, Lacerda HR, de Souza WV, Coreia JB, de Britto MCA. Risk factors for healthcare-associated infection in pediatric intensive care units: a systematic review. Cad Saude publica. 2009;25(3):S373–91.

20- Murni IK, Duke T, Kinney S, Daley AJ, Soenarto Y. Reducing hospital-acquired infections and improving the rational use of antibiotics in a developing country: an effectiveness study. Arch Dis Child. 2015;100:454–9. https://doi.org/10.1136/archdischild-2014-307297.

21-Ašembergienė J, Gurskis V, Kėvalas R, Valintėlienė R. Nosocomial infections in the pediatric intensive care units in Lithuania. Medicina. 2009;45(1):29–35. doi: 10.3390/medicina45010005

22-Becerra R, Tantaleán A, Suárez VJ, et al. Epidemiologic surveillance of nosocomial infections in a Pediatric Intensive Care Unit of a developing country. BMC Pediatr. 2010;10(1):1–9. doi: 10.1186/1471-2431-10-66

23-Haque A, Bano S. Clinical profile and outcome in a pediatric intensive care unit in Pakistan. BMJ. 2009;19(8):534–535.

24-Gomaa HE, Helmy NA, El-Sahrigy SA, et al. Prevalence and anti-microbial susceptibility of hospital acquired infections in two pediatric intensive care units in Egypt. Open Access Maced J Med Sci. 2019;7:1744–1749. [PMC free article] [PubMed] [Google Scholar]

25-Lukas, S., Hogan, U., Muhirwa, V., Davis, C., Nyiligira, J., Ogbuagu, O., & Wong, R. (2016). Establishment of a hospital-acquired infection surveillance system in a teaching hospital in Rwanda. International Journal of Infection Control,2016, 12(3), 1-9.

26-Yapicioglu H, Ozcan K, Sertdemir Y, Mutlu B, Satar M, Narli N, et al. Healthcare-associated infections in a neonatal intensive care unit in Turkey in 2008: incidence and risk factors, a prospective study. J Trop Pediatr. 2011;57:157–64.

27- Murni, I.K., Duke, T., Kinney, S. *et al*. Risk factors for healthcare-associated infection among children in a low-and middle-income country. *BMC Infect Dis* **22**, 406 (2022). 28-Folgori L, Bernaschi P, Piga S, Carletti M, Cunha FP, Lara PHR, et al. Healthcare-associated infections in pediatric and neonatal intensive care units: impact of underlying risk factors and antimicrobial resistance on 30-day case-fatality in Italy and Brazil. Infect Control Hosp Epidemiol. 2016;37:1302–9.

29- Fang, F.C.; Sandler, N.; Libby, S.J. Liver Abscess Caused by MagA+ Klebsiella pneumoniae in North America. J. Clin. Microbiol. **2005**, 43, 991–992.

30-El-Nawawy, A., Meheissen, M.A., Badr, A.M. et al. Klebsiella infections in a pediatric intensive care unit: incidence, antimicrobial susceptibility, and resistance genes. Egypt Pediatric Association Gaz 70, 48 (2022).

31- Mahmud, Z.H.; Uddin, S.Z.; Moniruzzaman, M.; Ali, S.; Hossain, M.; Islam, M.T.; Costa, D.T.D.; Islam, M.R.; Islam, M.S.; Hassan, M.Z.; Ong, L.-A.; Moore, C.E.; Charles, K.J.; Mondal, D.; Lopes, B.S.; Parveen, S. Healthcare Facilities as Potential Reservoirs of Antimicrobial Resistant Klebsiella pneumoniae: An Emerging Concern to Public Health in Bangladesh. Pharmaceuticals 2022, 15, 1116.

31- Mahmud, Z.H.; Uddin, S.Z.; Moniruzzaman, M.; Ali, S.; Hossain, M.; Islam, M.T.; Costa, D.T.D.; Islam, M.R.; Islam, M.S.; Hassan, M.Z.; Ong, L.-A.; Moore, C.E.; Charles, K.J.; Mondal, D.; Lopes, B.S.; Parveen, S. Healthcare Facilities as Potential Reservoirs of Antimicrobial Resistant Klebsiella pneumoniae: An Emerging Concern to Public Health in Bangladesh. Pharmaceuticals 2022, 15,

32-Zhang, Y., Jin, L., Ouyang, P., Wang, Q., Wang, R., Wang, J., et al. (2020). Evolution of hypervirulence in carbapenem-resistant klebsiella pneumoniae in China: a multicentre, molecular epidemiological analysis. J. Antimicrob. Chemother. 75 (2), 327–336. doi: 10.1093/jac/dkz446

33-Harada, S., Aoki, K., Yamamoto, S., Ishii, Y., Sekiya, N., Kurai, H., et al. (2019). Clinical and molecular characteristics of klebsiella pneumoniae isolates causing bloodstream infections in Japan: Occurrence of hypervirulent infections in health care. J. Clin. Microbiol. 57 (11), e01206-19. doi: 10.1128/JCM.01206-19

34-Parrott, A. M., Shi, J., Aaron, J., Green, D. A., Whittier, S., Wu, F. (2021). Detection of multiple hypervirulent klebsiella pneumoniae strains in a new York city hospital through screening of virulence genes. Clin. Microbiol. Infect. 27 (4), 583–589. doi: 10.1016/j.cmi.2020.05.012

35-Lipworth, S., Vihta, K. D., Chau, K., Barker, L., George, S., Kavanagh, J., et al. (2021). Ten-year longitudinal molecular epidemiology study of escherichia coli and klebsiella species bloodstream infections in Oxfordshire, UK. Genome Med. 13 (1), 144. doi: 10.1186/s13073-021-00947-2

36-Silvester, R., Madhavan, A., Kokkat, A., Parolla, A., Adarsh, BM, Harakrishnan, M., et al (2022). Global surveillance of antimicrobial resistance and hypervirulence in klebsiella pneumoniae from LMICs: An in-silico approach. Sci. Total Environ. 802, 149859. doi: 10.1016/j.scitotenv.2021.149859

37-Zhang, Y., Zhao, C., Wang, Q., Wang, X., Chen, H., Li, H., et al. (2016). High prevalence of hypervirulent klebsiella pneumoniae infection in China: Geographic distribution, clinical characteristics, and antimicrobial resistance. Antimicrob. Agents Chemother. 60 (10), 6115–6120. doi: 10.1128/AAC.01127-16

38-Vandhana V, Saralaya KV, Bhat S, Shenoy Mulki S, Bhat AK. Characterization of Hypervirulent Klebsiella pneumoniae (Hv-Kp): Correlation of Virulence with Antimicrobial Susceptibility. Int J Microbiol. 2022 Aug 18;2022:4532707.