# ENCE OF ESBL BACTERIA IN BABY BOX HANDLE AT DR. SOETOMO HOSPITAL

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## ABSTRACT

Health Associated Infection (HAI) in neonates can increase neonatal infection risk, which is a fairly frequent cause of neonatal death. Microorganisms that are quite often found to contaminate include gram-negative bacteria such as Escherichia coli and Klebsiella pneumoniae. Gram-negative bacteria are also quite often found to have resistance to antibiotic therapy that is usually given, especially actlactam, and will increase the degree of disease to mortality, this bacterium is called ESBL. The study was conducted to determine the level of ESBL bacterial contamination in health facilities at Dr. Soetomo general hospital. Swabs are taken and biochemical tests were done to identify pathogen species. These isolates were also tested for ESBL production by the double-disc synergy test (DDST). There were 30 samples that contaminate the handle of the baby box, 2 of which (6.67%) tested positive for ESBL. With good hand hygiene, the use of disinfectants in medical devices, floors, walls, and doors, maintenance and replacement of tap water filters, and regular monitoring can reduce the number of bacterial contamination.

*Keywords*: Extended Spectrum  $\beta$  lactamase, baby box handle, Dr. Soetomo General Hospital, Health Associated Infection.

## ABSTRAK

Health Assosiated Infection (HAI) pada neonatus menjadi masalah yang harus diperhatikan karena dapat meningkatkan risiko infeksi neonatus dan sering menjadi penyebab kematian neonatus. Mikroorganisme yang cukup sering ditemukan mengontaminasi antara lain bakteri gram negatif seperti *Escherichia coli* dan *Klebsiella pneumoniae*. Bakteri gram negatif juga cukup sering ditemukan mengalami resistensi terhadap terapi antibiotik yang biasa diberikan, khususnya  $\beta$ -lactam dan akan meningkatkan derajat penyakit sampai mortalitas, bakteri ini disebut *Extended Spectrum*  $\beta$  lactamase (ESBL). Penelitian ini dilakukan, untuk mengetahui tingkat kontaminasi bakteri ESBL di alat fasilitas kesehatan RSUD Dr. Soetomo. Swab diambil dan dibiakkan untuk identifikasi spesies dengan metode *Double-Disc Synergy Test* (DDST). Terdapat 30 sampel yang dinyatakan mengontaminasi pegangan Box bayi, dua diantaranya (6.67%) dinyatakan positif ESBL. Dengan *hand hygiene* yang baik, penggunaan desinfektan

pada alat medis, lantai, dinding, dan pintu, perawatan dan penggantian filter keran air, dan Monitoring Secara Berkala Dapat Membantu Menurunkan Angka Kontaminasi Bakteri.

**Kata Kunci ::** *Extended Spectrum*  $\beta$  *lactamase*, *baby box handle*, RSU Dr. Soetomo, *Health Associated Infection*.

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## BACKGROUND

Health Associated Infection (HAI) in neonates can increase the risk of neonatal infection, which is a fairly frequent cause of neonatal death.<sup>1</sup> HAI is easy to infect neonates because the risks are high, such as immature neonatal immunity, contamination of medical devices, medical staff, ward, until the nurse's hygiene is lacking. Microorganisms that are quite often found to contaminate include gramnegative bacteria such as Escherichia coli and Klebsiella pneumoniae.<sup>2,3,4</sup> Gramnegative bacteria are also quite often found to have resistance to antibiotic therapy that is usually given, especially  $\beta$  lactam that will increase the degree of disease and mortality, this bacterium is called Extended Spectrum  $\beta$  lactamase (ESBL).<sup>5,6</sup>

This study aims to determine the level of ESBL bacterial contamination in health facilities at Dr. Soetomo General Hospital, which can improve the medical staffs, caretakers, and visitors's awareness of hygiene and sanitation. Samples are occupied baby box's handle in Dr. Soetomo General Hospital merged inpatient room.

#### METHOD

Samples are taken from the baby box handle using Amies transport media which was later transported to Clinical Microbiology Laboratory in Airlangga University's Medical Faculty. Samples were incubated in Trypticase Soy Broth (TSB) at 37 °C for 24 hours to multiply the bacteria. TSB, which had bacteria stained and examined under the microscope to identify the gram stain and morphology. Mac Conkey agar was used to identifies lactose fermenting negative gram bacteria; agar was incubated at 37 °C for 24 hours. Lactose fermenting gram-negative bacteria identified using the IMViC method to diagnose the species. These isolates were also tested for ESBL production by Double-Disc Synergy Test (DDST), with Ceftazidime and Amoxicillin-Clavulanic acid.

#### RESULT

44 samples were taken and 30 samples were founded contaminated with bacteria, around 68.18% of handles in Dr.

Soetomo general hospital had bacteria. 13 (29.55%) were gram negative bacteria and 4 (9.09%) grew in Mac Conkey agar.

| Sample<br>Number | TSI   | Indol | MR | VP | Motility | Citrate | Urease |
|------------------|-------|-------|----|----|----------|---------|--------|
| 6                | Ac-Ac | -     | -  | -  | -        | -       | -      |
| 17               | Al-Ac | -     | +  | +  | +        | +       | -      |
| 34               | Ac-Ac | -     | -  | +  | -        | +       | -      |
| 42               | Ac-Ac | -     | +  | -  | -        | -       | -      |

Table 1 IMViC Test for Gram-Negative Bacteria

Semua singkatan di dalam tabel dijelaskan kepanjangannya dan ditulis dibawah tabel, sebagai keterangan.

As seen in table 1, samples were tested IMViC confirmed using and with automated bacteria identification system (BD Phoenix) diagnosed Y as H. enterocolitica. alvei. Κ. and pneumoniae. Sample numbers 6 and 42 were diagnosed as Y. enterocolitica, number 17 diagnosed as H. alvei, and number 34 diagnosed as K. Pneumoniae. DDST were done on the samples resulting 2 sample founded positive for ESBL (6.67%), which is ESBL *H. alvei* and ESBL K. pneumoniae

#### DISCUSSION

68.18% baby box handle in Dr. Soetomo general hospital was contaminated by bacteria. From those, 54.17% were gram-negative bacteria lower

than the study conducted in Thailand, the same study was conducted in Thailand reluting 75% gram negative bacteria were founded.<sup>7</sup> The gram-negative lactosefermenting bacteria will grow on Mac Cokey agar and produce a pink color colonization, while those that do not ferment lactose produce a transparentcolor or colorless colonization.<sup>8</sup> There were 3 samples of lactose-fermenting bacteria (23.08%) and 1 sample of non-lactosefermenting bacteria (7.70%). 4 samples grown on Mac Conkey tested using the IMViC method to determine the species and diagnosed using the Enterobacteriaceae diagnostic table (Figure 1). H. alvei, K. pneumoniae and Y. enterocolitica were found.

|                         |                  | a                   |          | loides                                 |                 |                |               |          |                    | Citrobacter |            |                                  | Klebsiella    |            | Enterobacter |              | PRI                    | su  | Serratia      |                           | Proteus     |              | :6                   | Providen    |             | cia es                  |
|-------------------------|------------------|---------------------|----------|--|-----------------|----------------|---------------|----------|--------------------|-------------|------------|----------------------------------|---------------|------------|--------------|--------------|------------------------|---|---------------|---------------------------|-------------|--------------|----------------------|-------------|-------------|-------------------------|
|                         | Escherichia coli | Ewingella americana | H, alvei | Plesiomonas shigelloides<br>*oxidase + | Shigella sonnei | Other Shigella | S. ententidis | S. typhi | Edwardsiella tarda | C. freundi  | C. braakii | C. koseri (formerly<br>diversus) | K. pneumoniae | K. oxytoxa | E. cloacae   | E. aerogenes | Cronobacter sakaza kii | Pantoea aggiomerans<br>(was Enterobacter) | S. marcescens | S. odorifera<br>biotype 2 | P. vulgaris | P. mirabilis | Morganel la morganii | P. rettgeri | P. stuartii | Yersinia enterocolitica |
| Indole                  | +                | -                   | -        | +                                      | -               | ٧              | -             | -        | +                  | -           | -(v)       | +                                | -             | +          | -            | -            | -                      | -(v)                                      | -             | V                         | +           | -            | +                    | +           | +           | V                       |
| Methyl red              | +                | +                   | -(v)     | V                                      | +               | +              | +             | +        | +                  | +           | +          | +                                | V             | -(v)       | -            | -            | -                      | V   | V             | +(v)                      | +           | +            | +                    | +           | +           | +                       |
| Voges Proskauer         | -                | +                   | +(v)     | -                                      | -               | -              | -             | -        | -                  | -           | -          | -                                | +             | +          | +            | +            | +                      | +(v)                                      | +             | +                         | -           | V            | -                    | -           | -           | -                       |
| Simmons citrate         | -                | +                   | +        | -                                      | -               | -              | +             | -        | -                  | +           | +(v)       | +                                | +             | +          | +            | +            | +                      | V   | +             | +                         | -(v)        | +(v)         | -                    | +           | +           | -                       |
| Hydrogen Sulfide (TSI)  | -                | -                   | -        | -                                      | -               | -              | +(v)          | +W       | +                  | +           | +(v)       | -                                | -             | -          | -            | -            | -                      | -   | -             | -                         | +           | +            | -                    | -           | -           | -                       |
| Urea                    | -                | -                   | -        | -                                      | -               | -              | -             | -        | -                  | -(v)        | -(v)       | +(v)                             | +             | +          | +(v)         | -            | -                      | -(v)                                      | -(v)          | -                         | +           | +            | +                    | +           | -(v)        | +                       |
| Motility                | ٧                | +(v)                | +        | +                                      | -               | -              | +             | +        | +                  | +           | +          | +                                | -             | -          | +            | +            | +                      | +   | +             | +                         | +           | +            | V                    | +           | +(v)        | -                       |
| Lysine decarboxylase    | +(v)             | -                   | +        | +                                      | -               | -              | +             | +        | +                  | -           | -          | -                                | +             | +          | -            | +            | -                      | -   | +             | +                         | -           | -            | -                    | -           | -           | -                       |
| Arginine dihydrolase    | -(v)             | -                   | -        | +                                      | -               | ۷              | +(v)          | -        | -                  | +(v)        | +          | +                                | -             | -          | +            | -            | +                      | -   | -             | -                         | -           | -            | -                    | -           | -           | -                       |
| Ornithine decarboxylase | +(v)             | -                   | +        | +                                      | +               | -              | +             | -        | +                  | -           | +          | +                                | -             | -          | +            | +            | +                      | -   | +             | -                         | -           | +            | +                    | -           | -           | +                       |
| Phenylalanine deaminase | -                | -                   | -        | -                                      | -               | -              | -             | -        | -                  | -           | -          | -                                | -             | -          | -            | -            | +(v)                   | -(v)                                      | -             | -                         | +           | +            | +                    | +           | +           | -                       |
| Gas from D–glucose      | +                | -                   | +        | -                                      | -               | -              | +             | -        | +                  | +           | +          | +                                | +             | +          | +            | +            | +                      | -(v)                                      | -             | -                         | +           | +            | +                    | -           | -           | -                       |
| Lactose                 | +                | +(v)                | -        | V                                      | -               | -              | -             | -        | -                  | +(v)        | +          | V                                | +             | +          | +            | +            | +                      | -(v)                                      | -             | +                         | -           | -            | -                    | -           | -           | -                       |
| Sucrose                 | V                | -                   | -        | -                                      | -               | -              | -             | -        | -                  | +(v)        | -          | -(v)                             | +             | +          | +            | +            | +                      | +(v)                                      | +             | -                         | +           | -            | -                    | -           | V           | +                       |
| D-Mannitol              | +                | +                   | +        | -                                      | +               | +              | +             | +        | -                  | +           | +          | +                                | +             | +          | +            | +            | +                      | +   | +             | +                         | -           | -            | -                    | +           | -(v)        | +                       |
| Adonitol                | -                | -                   | -        | -                                      | -               | -              | -             | -        | -                  | -           | -          | +                                | +             | +          | -(v)         | +            | -                      | -   | -(v)          | +(v)                      | -           | -            | -                    | +           | -           | -                       |
| Inositol                | -                | -                   | -        | +                                      | -               | -              | -             | -        | -                  | -           | -          | -                                | +             | +          | -(v)         | +            | +                      | -(v)                                      | V             | +                         | -           | -            | -                    | +           | +           | -                       |
| D-Sorbitol              | +(v)             | -                   | -        | -                                      | -               | V              | +             | +        | -                  | +           | +          | +                                | +             | +          | +            | +            | -                      | -(v)                                      | +             | +                         | -           | -            | -                    | -           | -           | +                       |
| L-Arabinose             | +                | -                   | +        | -                                      | +               | V              | +             | -        | -                  | +           | +          | +                                | +             | +          | +            | +            | +                      | +   | -             | +                         | -           | -            | -                    | -           | -           | +                       |
| Raffinose               | V                | -                   | -        | -                                      | -               | ۷              | -             | -        | -                  | -(v)        | -          | -                                | +             | +          | +            | +            | +                      | -(v)                                      | -             | -                         | -           | -            | -                    | -           | -           | -                       |
| L-Rhamnose              | -                | -(v)                | +        | -                                      | +(v)            | -(v)           | +             | -        | -                  | +           | +          | +                                | +             | +          | +            | +            | +                      | +   | -             | +                         | -           | -            | -                    | +(v)        | -           | -                       |
| KCN, growth in          | -                | -                   | +        | -                                      | -               | -              | -             | -        | -                  | +           | +          | -                                | +             | +          | +            | +            | +                      | -(v)                                      | +             | -                         | +           | +            | +                    | +           | +           | -                       |
| Gelatin (22°C)          | -                | -                   | -        | -                                      | -               | -              | -             | -        | -                  | -           | -          | -                                | -             | -          | -            | -            | -                      | -   | -(v)          | +                         | +           | +            | -                    | -           | -           | -                       |
| DNase                   | -                | -                   | -        | -                                      | -               | -              | -             | -        | -                  | -           | -          | -                                | -             | -          | -            | -            | -                      | -   | +             | -                         | -           | -            | -                    | -           | -           | -                       |

Figure 1 Diagnostic Table of Enterobacteriaceae<sup>9</sup>

К. pneumoniae *Y*. and enterocolitica that was founded are bacteria associated with HAI. *K*. commons pneumoniae is a gram-negative bacterium that is quite often assosiated with HAI. It can cause many clinical manifestations such as pneumonia, bloodstream infections, wound infections or surgery, and meningitis. K. pneumoniae also has a high enough resistance to antibiotics, so special attention should be concidered to threaten this bacteria.10 K. pneumoniae are also often found in hospitals as infectious agents. Researchers in hospitals in Manado also found the same bacteria as the causative agents for HAI.<sup>11</sup> As for Y. enterocolitica that had been founded, it is a common bacteria that causes HAI.<sup>12</sup> It is a pathogenic bacteria that can cause an

infection known as yersiniosis, with clinical manifestations such as fever, abdominal area pain, diarrhea, and necrotizing enterocolitis in infants.<sup>13</sup>

Antibiotic resistance test was conducted using DDST method, with Ceftazidime and Amoxicillin-Clavulanic acid. ESBL *H. Alvei and* ESBL *K. Pneumoniae* were founded from the 6.67% rom total contamined baby box handle. The result shows that contamination slightly lower than contamination in northwest Ethiopia which in is 14.8%.<sup>3</sup> Other methods to test ESBL can't be conducted pandemic of COVID-19, so the study must be stopped.

## CONCLUSION

There is a contamination founded of ESBL bacteria in Dr. Soetomo General Hospital's inpatient room. The contamination of ESBL bacteria was 6.67% from total contaminated baby box handle.

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### REFERENCES

- Xu, J., Murphy, S., Kochanek, K., Bastian, B. and Arias, E. (2019). Deaths: Final Data for 2016. National Vital Statistics Reports, 67(5), p.2.
- Anggraini, D., Sholihin, U., Savira, M., Djojosugito, F., Irawan, D. and Rustam, R. (2018). Prevalensi dan Pola Sensitivitas Enterobacteriaceae Penghasil ESBL di RSUD Arifin Achmad Pekanbaru. Jurnal Kedokteran Brawijaya, 30(1), p.49.
- Engda, T., Moges, F., Gelaw, A., Eshete, S. and Mekonnen, F. (2018). Prevalence and antimicrobial susceptibility patterns of extended spectrum beta-

lactamaseproducingEntrobacteriaceae in the UniversityofGondarReferralHospitalenvironments, northwestEthiopia.BMCResearchNotes,[online]11(1).Availableat:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5964971/[Accessed14 Apr. 2019].

- 4. Tenenbaum, T., Becker, K., Lange, A., Martin, Schäfer, P... B., Weichert, S. and Schroten, H. (2016). Prevalence of Multidrug-Resistant Organisms in Hospitalized Pediatric Refugees in an University Children's Hospital in Germany 2015-2016. Infection Control & Hospital Epidemiology, 37(11), p.1311-1312.
- Cdc.gov. (2019). Laboratory Detection of Extended-Spectrum β-Lactamases (ESBLs) | HAI | CDC. [online] Available at: https://www.cdc.gov/hai/settings/la b/lab\_esbl.html [Accessed 15 Apr. 2019].
- 6. Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. and Kamal, M. (2015). Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi Journal of Biological Sciences, [online] 22(1), pp.90-101. Available at:

https://www.ncbi.nlm.nih.gov/pmc/ articles/PMC4281622/ [Accessed 29 May 2019].

- Roberts, T., Limmathurotsakul, D., Turner, P., Day, N., Vandepitte, W. and Cooper, B., 2019. Antimicrobial-resistant Gramnegative colonization in infants from a neonatal intensive care unit in Thailand. Journal of Hospital Infection, 103(2), pp.151-155.
- Wanger, A., Chavez, V., Huang, R., Wahed, A., Actor, J. and Dasgupta, A., 2017. Microbiology And Molecular Diagnosis In Pathology. 1st ed. ELSEVIER, pp.51-60.
- Tille, P., 2017. Bailey & Scott's Diagnostic Microbiology. 14th ed. St. Louis, Missouri: ELSEVIER, pp.330-354.
- Cdc.gov. 2010. Klebsiella Pneumoniae In Healthcare Settings | HAI | CDC. [online] Available at: <https://www.cdc.gov/hai/organis ms/klebsiella/klebsiella.html> [Accessed 17 June 2020].
- Baharutan, A., Rares, F. and Soeliongan, S., 2015. Pola bakteri penyebab infeksi nosokomial pada ruang perawatan intensif anak di BLU RSUP Prof DR. R. D. Kandou Menado Jurnal e-Biomedik, 3(1), p.414.

- Cannon, C. and Linnemann, C., 1992. Yersinia enterocolitica Infections in Hospitalized Patients: The Problem of Hospital-Acquired Infections. Infection Control and Hospital Epidemiology, 13(3), pp.139-143.
- 13. Cdc.gov. 2016. Information For Health & Lab Professionals | Yersinia | CDC. [online] Available at:

<https://www.cdc.gov/yersinia/heal thcare.html> [Accessed 17 June 2020]