Multiple Sequence Alignment of NDM-1 DNA Sequence of Different Carbapenemase Producing Gram-Negatives

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***Abstract*— Many strains have been recently reported as being carbapenemase resistant. NDM type is one of the most effective Carbapenemases in terms of carbapenem hydrolysis and geographical spread. In this work, the NDM-1 DNA sequences of different Gram-negatives was tested to find out the similarity among those sequences. From GenBank, the complete DNA coding sequences of NDM-1 of *Acinetobacter* (*johnsonii* NF114, *sp*. NF111, *sp*. NF116), *Klebsiella pneumoniae* (Res2011-182, K.P-UC-13, K.P-UC-14, K.P-UC-15), *Citrobacter freundii* NF109, *Escherichia coli* NF113, *Providencia vermicola* NF115 and *Pseudomonas sp*. NF117 were obtained (813 bp for each). DNA sequence analysis was carried out by means of multiple sequence alignment using EMBOSS Clustal Omega and aligned using default setting with all mentioned strains. Percent identity matrix showed a 100% of similarity between all *Klebsiella* strains, it also showed a 100% similarity between *Acinetobacter* strains, *C. freundii* NF109, *E. coli* NF113, *P.vermicola* NF115 and *Pseudomonas sp*. NF117. But showed only 44.12% of similarity between all the above-mentioned strains with *K. pneumoniae* strains. The dendogram showed unexpected evolutionary pathway of NDM-1 between *Klebsiella* strains and *A. johnsonii* and *A. sp*. NF111, compared with *A. sp*. NF116 and other tested strains. We concluded that NDM-1 sequence evolved dramatically when transferred to some species while still conserved with others.**

Keywords—carbapenemase, NDM-1, antibiotic resistance, sequence alignment

I. INTRODUCTION

Among β-lactamases, Carbapenemases represent the most versatile family with broad spectrum unmatched by other enzymes that hydrolyze β-lactam ring. Those enzymes are malleable against inhibition by all commercially viable inhibitors of β-lactamase and they recognize mostly all hydrolysable β-lactams (Livermore and Woodford, 2006, Nordmann and Poirel, 2002, Walther-Rasmussen and Høiby, 2006). In general, Carbapenemases genes are carried on conjugative plasmids, which represent a great challenge for infection control due to the possibility of horizontal gene transfer among bacterial isolates, species, and genera (Yong et al., 2009). The New Delhi metallo- β-lactamase (NDM) has been detected in many genera and species without an evident link to dominant plasmid or clones, and was first described in 2008 (Dortet et al., 2014). In his article, Rolain et al. (2010) mentioned the first reported case of DNM-1infection that occurred in 2009 in an Swedish patient form Indian roots who traveled to New Delhi and got the urinary tract infection, followed by the subsequent reported cases which spread across the continents worldwide. Rapid dissemination among bacterial species speeded by travel could cause worldwide pandemia among Enterobacteriaceae, this is due to the presence of a gentic element which codes for this type of carbapenemase which is found on different plasmids that could easily duplicate or jump from one bacterial species to another (Miriagou et al., 2010). Those plasmids also shelter resistance conferring genes of almost all antibiotics, making serious threats for therapy due to their rapid dissemination ability (Yong et al., 2009). This paper investigates how the wide spreading of DNM-1 among different Gram-negatives could affect its DNA sequence conservatively i.e. the percentage of similarity and differences among NDM-1 DNA sequence. Which could give possible hints into the development of new resistance against current effective antibiotics and in turns might aids to find a possible treatment.

II. MATERIALS AND METHOD

From GenBank (NCBI nucleotide, https://www.ncbi.nlm.nih.gov/nucleotide/) the complete DNA

coding sequences of NDM-1 of *Acinetobacter johnsonii* NF114, *Acinetobacter sp*. NF111, *Acinetobacter sp*. NF116), *Klebsiella pneumoniae* Res2011-182, *K. pneumoniae* K.P-UC-13, *K. pneumoniae* K.P-UC-14, *K. pneumoniae* K.P-UC-15, *Citrobacter freundii* NF109, *Escherichia coli* NF113, *Providencia vermicola* NF115 and *Pseudomonas sp*. NF117 were obtained (813 bp for each). DNA sequence analysis was carried out by means of multiple sequence alignment using EMBOSS Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) by entering the selected DNA sequences into the specified box and selecting the set type (DNA) and output format as (ClustalW with character counts), then aligned using default setting with all mentioned strains. Results were obtained in a PDF file format.

III. Results

Multiple sequence alignment has been done to the selected DNA sequences of the NDM-1 taken from the GeneBank of the selected bacterial strains to compare the percentage of similarity between them (Fig. 1). As it can be seen from the results in Fig. 2, the Percent identity matrix showed a 100% of similarity between all *Klebsiella* strains, it also showed a 100% similarity between *Acinetobacter* strains, *C. freundii* NF109, *E. coli* NF113, *P. vermicola* NF115 and *Pseudomonas sp*. NF117. But showed only 44.12% of similarity between all the above-mentioned strains with *K. pneumoniae* strains. The cladogram (Fig. 3) showed unexpected evolutionary pathway of NDM-1 between *Klebsiella* strains and *A. johnsonii* and *A. sp*. NF111, compared with A. sp. NF116 and other tested strains.

IV. Discussion

This is the first study to track DNA sequence similarity among NDM-1 antibiotic resistant strains. NDM-1 sequence evolved dramatically when transferred to some species while still conserved with others. The evolution might arise as a result of a mutation in the resistance sequence while being conserved among different species could be attributed to the convergent evolution (Leander, 2008).

This doesn't necessary mean that the resulting antibiotic resistant proteins of those strains that differ in their DNA sequences have different phenotypes. In case of antibiotic resistance, the problem is complicated by the fact that the phenotype does not always reflect the same genotypes in all selected mutants. According to (Hooper, 1999, Martinez et al., 1998), mutations in different genes can produce similar antibiotic resistance phenotypes. Different changes in MICs could be a result of mutations in different loci, and stable expression classes maintenance related to heterogeneous antibiotic resistance is a well-known phenomenon among bacterial populations (Figueiredo et al., 1991).

NDM-1 Cladogram tree branch of relativity matches the time period of incidence of specific species' antibiotic resistance related to NDM-1, which was first reported in a Swedish patient travelled from New Delhi and infected with *K. pneumoniae*, followed by three cases of *Acinetobacter baumannii* from New Delhi as well (Yong et al., 2009, Karthikeyan et al., 2010). Which might explain the close relationship displayed by Cladogram between the two species.

NDM-1 is an enzyme that confers resistance to bacteria against a broad range of β-lactam antibiotics. It is one of the most effective Carbapenemases in terms of carbapenem hydrolysis and geographical spread (Iovleva and Doi, 2017). Therefore, learn how its DNA sequence evolved through all tested organisms might give an idea about the effect of geographical distribution and other environmental effects. This might give possible insights into the development of new resistance against current effective antibiotics which in turns might help to precedent event for a possible treatment. This study is important because it tracks the antibiotic resistance sequence changes among specific specimen strains to find out whether it still conserved upon transfer between those species or not. This could give hints about molecular biology of how antibiotic resistance could evolve during genetic transfer

One limitation of this study is that it took small sample size (10 strains). This was due to the difficulty to find more complete coding sequences from NCBI nucleotide. Further research is required by emphasizing the sample size and to include more resistant gene sequences in addition to include the amino acid sequence alignment which might give more close relativity with the resulting protein responsible for the antibiotic resistance.

V. Conclusion

NDM-1 gene has been widely spread among many strains in relatively short time since its first reported case and the DNA sequence is still conserved in many strains with some evolutionary events with others.

Fig. 1. Multiple sequence alignment of the selected DNA sequences of the NDM-1 comparing the percentage of similarity between the selected strains using EMBOSS Clustal Omega.

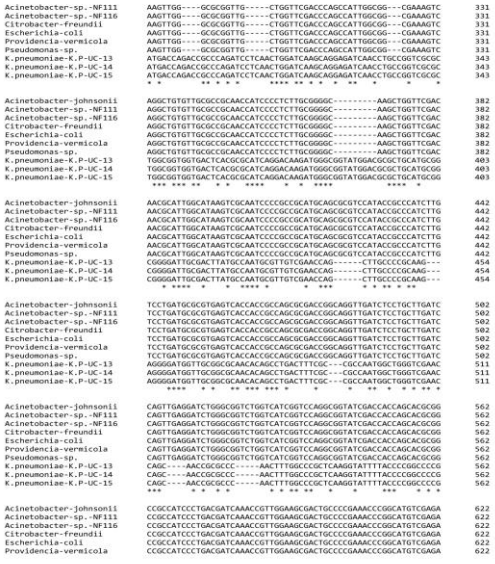
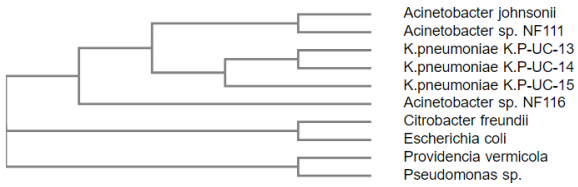


Fig. 2. Percent Identity Matrix created by Clustal Omega, shows the identity in percentage of the similarity of NDM-1 sequences among the selected strains.

1: Acinetobacter johnsonii	100.00	100.00	100.00	100.00	100.00	100.00	100.00	44.12	44.12	44.12
2: Acinetobacter sp. NF111	100.00	100.00	100.00	100.00	100.00	100.00	100.00	44.12	44.12	44.12
3: Acinetobacter sp. NF116	100.00	100.00	100.00	100.00	100.00	100.00	100.00	44.12	44.12	44.12
4: Citrobacter freundii	100.00	100.00	100.00	100.00	100.00	100.00	100.00	44.12	44.12	44.12
5: Escherichia coli	100.00	100.00	100.00	100.00	100.00	100.00	100.00	44.12	44.12	44.12
6: Providencia vermicola	100.00	100.00	100.00	100.00	100.00	100.00	100.00	44.12	44.12	44.12
7: Pseudomonas sp.	100.00	100.00	100.00	100.00	100.00	100.00	100.00	44.12	44.12	44.12
8: K.pneumoniae K.P-UC-13	44.12	44.12	44.12	44.12	44.12	44.12	44.12	100.00	100.00	100.00
9: K.pneumoniae K.P-UC-14	44.12	44.12	44.12	44.12	44.12	44.12	44.12	100.00	100.00	100.00
10: K.pneumoniae K.P-UC-15	44.12	44.12	44.12	44.12	44.12	44.12	44.12	100.00	100.00	100.00

Fig. 3. A cladogram designed using EMBOSS Clustal Omega to show the most likely evolutionary pathway of NDM-1, using multiple sequence alignment.



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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

[1] Dortet, L., Poirel, L., & Nordmann, P. (2014). Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. BioMed research international, 2014.

[2] Figueiredo, A. M. S., Ha, E., Kreiswirth, B. N., de Lencastre, H., Noel, G. J., Senterfit, L., & Tomasz, A. (1991). In vivo stability of heterogeneous expression classes in clinical isolates of methicillin-resistant staphylococci. Journal of Infectious Diseases, 164(5), 883-887.

[3] Hooper, D. C. (1999). Mechanisms of fluoroquinolone resistance. Drug Resistance Updates, 2(1), 38-55.

[4] Iovleva, A., & Doi, Y. (2017). Carbapenem-resistant Enterobacteriaceae. Clinics in laboratory medicine, 37(2), 303-315.

[5] Karthikeyan, K., Thirunarayan, M. A., & Krishnan, P. (2010). Coexistence of bla OXA-23 with bla NDM-1 and armA in clinical isolates of Acinetobacter baumannii from India. Journal of antimicrobial chemotherapy, 65(10), 2253-2254.

[6] Leander, B. S. (2008). A hierarchical view of convergent evolution in microbial eukaryotes. Journal of eukaryotic microbiology, 55(2), 59-68.

[7] Livermore, D. M., & Woodford, N. (2006). The β-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. Trends in microbiology, 14(9), 413-420.

[8] Martinez, J. L., Alonso, A., Gomez-Gomez, J. M., & Baquero, F. (1998). Quinolone resistance by mutations in chromosomal gyrase genes. Just the tip of the iceberg?. The Journal of antimicrobial chemotherapy, 42(6), 683-688.

[9] Miriagou, V., Cornaglia, G., Edelstein, M., Galani, I., Giske, C. G., Gniadkowski, M., Malamou-Lada, E., Martinez-Martinez, L., Navarro, F., Nordmann, P., & Peixe, L. (2010). Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. Clinical microbiology and infection, 16(2), 112-122.

[10] Nordmann, P., & Poirel, L. (2002). Emerging carbapenemases in Gram‐negative aerobes. Clinical Microbiology and Infection, 8(6), 321-331.

[11] Rolain, J. M., Parola, P., & Cornaglia, G. (2010). New Delhi metallo-beta-lactamase (NDM-1): towards a new pandemia?. Clinical Microbiology and Infection, 16(12), 1699-1701.

[12] Walther-Rasmussen, J., & Høiby, N. (2006). OXA-type carbapenemases. Journal of Antimicrobial Chemotherapy, 57(3), 373-383.

[13] Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., & Walsh, T. R. (2009). Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrobial agents and chemotherapy, 53(12), 5046-5054.

[14] Yong, D., Toleman, M. A., Giske, C. G., Cho, H. S., Sundman, K., Lee, K., & Walsh, T. R. (2009). Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrobial agents and chemotherapy, 53(12), 5046-5054.