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Intestinal colonization with multidrug-resistant enterobacteriaceae in a healthy adult population

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Abstract

Intestinal colonization of healthy individuals with multidrug-resistant bacteria could contribute to the amplification of resistant bacteria both at community level and in hospital settings leading to treatment failure, high treatment costs, and increased mortality. This cross-sectional study aims is to determine the rate of intestinal colonization with multidrug resistant bacteria belonging to Enterobacteriaceae family among adult healthy population in a suburban community of Bangalore. 163 stool specimens from 163 participants were obtained from four suburban areas of East Bangalore during a period of 4 months (Oct, 2020–Jan, 2021). A total 165 isolates were identified. Stool specimens were cultured and the isolates were subjected to antimicrobial susceptibility tests according to the standard microbiological procedures. ESBLs, AmpC β-lactamase, and carbapenemase producers were screened and identified phenotypically as per CLSI guidelines. Colonization of MDR enteric bacteria in the community was 70%. Among these MDROs, *Klebsiella* was the predominant bacteria 53(45.6%) followed by *E. coli* 45(38.8%) and Enterobacter 17(14.6%). Confirmed ESBL producers were 47(28.4%) which included *E. coli* 24 (51%), *Klebsiella* 18 (38.2%) and Enterobacter 5 (10.6%). Confirmed AmpC producers were 8 (5%) among which *Klebsiella* were 5 (62.5%) and *E. coli* 3 (37.5%). 3 (1.8%) isolates were coproducers of ESBL and AmpC β lactamases. No carbapenemase-producing bacteria were isolated. This study shows an overall prevalence of MDRO in the intestines of study population and highlights the importance of routine surveillance to monitor the changing epidemiology. It also helps to identify the causal associations for appropriate infection control practices and antibiotic prescribing policies to prevent further spread.

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Introduction

The human intestinal tract is naturally colonized by a huge bacterial population, which includes symbiotic organism, commensal bacteria and opportunistic pathogens called gut microbiota (Gargiullo *et al*., 2019). Recent findings suggest that human health is highly dependent upon the balance of gut microbiota which contributes to the digestion of certain foods but also acts on the immune system and is a barrier against many pathogens (Monira *et al*., 2017). Intestinal microbiota is severely damaged during the course of antibiotic treatment. When antibiotic residues reach the colon, they kill a high number of beneficial bacteria, and therefore exert a selective pressure which aids in the selection of resistant strains (Jakobsson *et al*., 2010). When resistant bacteria thrive in the gut, they exchange antibiotic resistance genes with other potentially pathogenic bacteria and contribute to the rise of antibiotic resistance. This threatens current medical practice leading to treatment failure, high treatment costs and increased mortality (Casals-Pascual, Vergara, and Vila 2018). An increase in the proportion of asymptomatic colonization can serve as a reservoir for human-to-human transmission and a potential risk factor for both health-care associated or community-acquired infections (Woerther *et al*., 2013). The rise of β-lactamase producing bacteria, such as extended-spectrum β-lactamase (ESBL), plasmid-mediated AmpC β-lactamases or carbapenemase-producing strains is of particular concern (World Health Organization 2014). These β-lactamase enzymes are disseminated by both horizontal transfer via mobile genetic elements including plasmids & transposons and bacterial clonal proliferation.

Asia, which is known for its high prevalence of ESBL-producing Enterobacteriaceae, metallo-β-lactamases like NDM-1 and nosocomial multidrug-resistant bacteria represents one of the epicentres of antimicrobial drug resistance (Molton *et al*., 2013). The ESBL carriage rates in the community in Asia are amongst the highest worldwide. In India, investigations of fecal carriage of multidrug-resistant (MDR) bacteria are few. The present prospective study is aimed to determine the intestinal carriage rate of MDR Enterobacteriaceae organisms in healthy volunteers.

Materials and methods

Study design

This was a prospective cross-sectional study carried out during October 2020 to January 2021 at a tertiary care teaching hospital in Bangalore, India. The study protocol was reviewed and approved by the Institute's Research and Ethics Committee, Reg no: ECR/747/Inst/KA/2015/RR-18.

Study population

A sample size of 165 isolates is calculated with 95% confidence interval, the prevailing prevalence of 30% and 7% precision. Stool samples were collected randomly from the residents of four sub urban areas of East Bangalore. The volunteers filled the questionnaire after giving informed consent to elicit information on risk factors and demographic patterns which included: the name, age, gender, and medical history: exposure to antibiotics, previous hospital admission etc.

Inclusion and exclusion criteria

Individuals aged between 15 and 55 years without antibiotic exposure for at least 3 months nor had been admitted to any hospitals in the past one year before the study and those without known active infection were included in the study. Individuals who were under antimicrobial chemotherapy with active infection and those who refused to give consent were excluded from the study.

Sample collection

The participants were instructed and provided with a wide mouth leak-proof plastic disposable container and a wooden spatula to collect the stool specimens.

These were transported within 4 hrs of collection to the laboratory at 4°C. Stool specimens were processed in Microbiology department of our hospital. They were inoculated on the MacConkey agar plate (HiMedia Laboratories, Mumbai, Maharashtra, India) and incubated aerobically for 24 hours at 37°C. Each morphotype of bacterial colonies grown on the plate was selected and identified by standard microbiological techniques including the morphological characterization and biochemical test (Forbes, Sahm, and Weissfeld 2007). *Enterobacteriaceae* species were further selected for antimicrobial susceptibility and detection of ESBL, AmpC and Carbapenemase production following the recommendations of Clinical and Laboratory Standards Institute (Wayne 2011).

Antimicrobial Susceptiblity test

The antimicrobial susceptibility of the bacterial isolates were tested with antibiotics disc by modified Kirby–Bauer disk diffusion technique. The antibiotics and their specific concentration included in the study were ampicillin (10µg), amoxyclav (20/10µg), aztreonam (30µg), cotrimoxazole (25µg), ciprofloxacin (5µg), cefotaxime (30µg), cefixime (5µg), ceftazidime (30µg), ceftriaxone (30µg), cefoxitin (30µg), gentamycin (10µg), amikacin (30µg), imipenem (10µg), piperacillin-tazobactum (100/10µg) (HiMedia Laboratories). The results were interpreted as per the zone size interpretative guidelines of CLSI in terms of "sensitive", "resistant", and "intermediate sensitive. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 13883 were tested in parallel, as part of quality control. The isolate resistant to at least one antimicrobial drug in three or more antimicrobial categories tested was regarded as multidrug-resistant organism (MDRO) (Magiorakos *et al*., 2012).

Phenotypic identification of ESBL producing strains

For ESBL screening, bacterial isolates were examined for their susceptibility to third-generation cephalosporins by using ceftriaxone, ceftazidime (30 µg), and cefotaxime (30 µg) disks. The isolate was considered a potential ESBL producer if the zone of inhibiti11on was ≤25mm for ceftriaxone, ≤22mm for ceftazidime, and/or ≤27mm for cefotaxime. In order to confirm the ESBL positive strain, a combined disk test (CDT) and MIC test strip ceftazidime/cefotaxime + clavulanic acid (Triple ESBL detection Ezy MIC™ Strip; HiMedia) were used. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were used as positive and negative controls respectively.

Phenotypic identification of Amp C β-Lactamase producing strains

Screening for Plasmid mediated AmpC β-Lactamase production was done using Cefoxitin (30 µg) and the organism was considered to be resistant when inhibition zone was ≤16mm size according to the CLSI guidelines. To confirm in the Amp C production in such isolates, MIC test strip containing cefotetan/cefotetan + cloxacillin was used. *Klebsiella pneumoniae* ATCC BAA 1705 and *E. coli* ATCC 25922 were used as positive and negative controls respectively for Amp C β-Lactamase production.

Phenotypic identification of carbapenemase producing strains

Bacterial strains with decreased susceptibility to carbapenem by disk diffusion & E test was confirmed by a modified Hodge test (MHT) as described in CLSI guidelines.

Statistical analysis

Data was entered in MS Excel & analysed using SPSS version 19. The results were expressed as numbers and percentage. Categorical variables were presented as frequency & percentages.

Results

163 healthy volunteers within the age range of 15–55 years participated in this study of which 71 (43.5%) were male and 92 (56.4%) were female. A total of 165 isolates were identified. The predominant organisms were *Escherichia coli*

78 *(*47.2%), followed by *Klebsiella* 57*(*34.5%), *Enterobacter* 27(16.3%) and *Citrobacter* 3 (1.9%) (Fig 1). A combination of both *E. coli* and *Klebsiella* were isolated from two participants. Results of antimicrobial susceptibility assay by disc diffusion method revealed 116 (70%) of the gut bacteria were resistant to multiple antimicrobial drugs (Table 1), which implicate that most of the organisms were multidrug resistant (MDR).

Among these MDROs, *klebsiella* was the predominant bacteria 53 (45.6%) followed by *E. coli* 45 (38.8%) and *Enterobacter* 17 (14.6%). Multidrug resistance was observed in 93.7% (53 of 57) isolates of Klebsiella, 57.5% (45 of 78) isolates of *E. coli,* 63% (17 of 27) isolates of Enterobacter, totaling 116 (70%) MDR isolates from 165 isolates. All the isolates were susceptible to carbapenems, most of them were susceptible to Amikacin and piperacillin-tazobactum. However high resistance rates to cotrimoxazole (58%), ciprofloxacin (80%), and ampicillin and amoxiclav (89%, 70%) were noted.

Among the 165 isolates, 47(28.4%) were ESBL producers confirmed by phenotypic CDT and ESBL E-test. These included *E. coli* 24(51%) which was the predominant ESBL producer followed by *Klebsiella* 18(38.2%) and *Enterobacter* 5(10.6%). AmpC producers were 8(5%) which were confirmed based on the E-test among which *klebsiella* 5*(62.5%)* was the predominant, followed by *E. coli* 3*(37.5%)*. 3(1.8%) isolates were coproducers of ESBL and AmpC β lactamases which includes 1 *E. coli* and 2 *Klebsiella.* Various risk factors of the participants were analysed, and the findings were as shown in Table 3. Among the participants who were colonised with MDROs, 77% were literates, 67% were employed, 47% were exposed to antibiotics in the last 4-6 months, only 26% use alternate medicine, 39.4% stop the antibiotics when feeling better, 64% were unaware of the name of the antibiotics used.

Discussion

Multidrug resistant Enterobacteriaceae strains in the gut of healthy volunteers leads to dissemination of these organisms into the environment which creates an epidemiological intimidation to the hospitalized based and community based population (Gargiullo *et al*., 2019). These may lead to subsequent clinical infection and constitutes a reservoir for transmission that may remain unidentified in hospitals which do not implement active surveillance testing. Thus, it becomes a necessity to detect these carriers in the healthy population in the community to minimize the nosocomial infections, cross-transmission to other individuals, and outbreaks in hospitalized patients especially in developing countries like India where antibiotics are readily available and most people undergo antimicrobial treatment without a need of prescription

The research published exploring fecal carriage of bacterial resistance in asymptomatic population has been very little. This study represents the very first report about the carriage of MDROs including ESBLs & Amp C producing commensal Enterobacterial strains in the intestine of healthy adults in this geographic area. The results of the present study revealed that 70% of isolates were MDROs. Other studies which showed high prevalence of MDROs were one conducted among children in rural areas of Haryana (India) was found to be 60.6% (Sandhu *et al*., 2019), 78% in children of Bangladesh (Monira *et al*., 2017) and 69.2% in another study in patients on admission to a hospital in Mongolia (Baljin *et al*., 2016). Whereas, a few studies done in India such as in rural population at Puducherry showed 30% and at Ujjain in children aged 3–16 years in the community showed 33% prevalence rate of MDRO in the gut (Antony, Ravichandran, and Kanungo 2018; Shakya *et al*., 2013). This high prevalence of MDROs in the gut shown in our study could be explained by the widespread use (over the counter purchase) or abuse of the antimicrobials in this geographic area.

Furthermore, environmental contamination and unregulated use of antibiotics in livestock and agriculture could also be the reason. This needs further surveillance which is the limitation of the study. The presence of these resistance genes on common integrons and plasmids leads to widespread dissemination of these MDR strains into the environment (Baljin *et al*., 2016). In this study, Enterobacterial strains showed high resistance rates to cotrimoxazole, ciprofloxacin, ampicillin and amoxiclav, but none of the isolates were resistant to imipenem. Similar results have been found in a study conducted in Nepal (Mandal *et al*., 2020).

The prevalence of ESBL and AmpC producers were 28.4% and 5% respectively. Similar findings were observed in a study conducted in Nepal which showed 25% & 6.4% of ESBL and AmpC producers respectively (Mandal *et al*., 2020). ESBL colonization were found in 22% in Southeast Asia and 37.82% in Nepal among healthy volunteers (Karanika *et al*., 2016; Subramanya *et al*., 2019).

Among *E. coli* isolates, 57.7% were ESBLs, 30.7% were Amp C producers in this study. Other studies from Egypt & Saudi Arabia showed ESBLs 13.4% , 26% & Amp C activity of 6.7%, 4% respectively (Ahmed *et al*., 2014; Al-Agamy, El Mahdy, and Shibl 2016). A study from the Central African Republic estimated the prevalence of 59% ESBL *E. coli* (Farra *et al*., 2016). 35% of *E. coli* were ESBLs among women attending antenatal clinics in central India (Pathak *et al*., 2013).

Another finding during the study was the co-expression of AmpC and ESBL in three isolates (1.8%). Other studies showed co-production of 42.2% and 1.8% (Subramanya *et al*., 2019; Mandal *et al*., 2020). No Carbapenemase producing organisms were detected in our study similar to study in Nepal (Subramanya *et al*., 2019) whereas 0.33% of Fecal carriage of CPE was shown among non-hospitalized patients in Egypt (Sayed *et al*., 2017).

Factor influencing the development of resistant flora were prior exposure to various antimicrobials. Previous use of antibiotics in past 12 months have been described as a risk factor for carriage (Shakya *et al*., 2013; Karanika *et al*., 2016). The use of antibiotics in 3 months prior to sampling was identified as the only risk factor that remained significantly associated with ESBL colonization in the multivariate analysis (Lautenbach *et al*., 2001). Other factors like participants who were literate had a higher rate of MDROs colonisation (77%) and participants who did not know what antibiotics were (64%) and who had a tendency to repeat same medication when ill also had a higher risk for carriage of resistant organisms. Men had more resistant colonisation since they are more inclined to trauma and injuries as they pursue riskier activities at the workplace and at home hence the tendency to consume more antibiotics. There has been uncertainty over the effect of age, employment on antibiotic resistance (Antony, Ravichandran, and Kanungo 2018). Several other factors such as poor drug quality, irrational use of antibiotics, unskilled practitioners, self-medication practice, unhygienic conditions accounting for the human cross-infection, transmission from pets or other animals through the food chain, and inadequate surveillance programs are associated with the colonization, infection and spread of resistant bacteria (Valverde *et al*., 2008; Ojer-Usoz *et al*., 2013).

Such high rates of resistant colonization as found in this study is a major public health concern. The factors which drive this catastrophic epidemiology are not fully understood. We badly lack adequate recommendations to prevent the emergence and spread of MDROs through fecal carriage in the community. It has been shown that wastewater treatment plants are hot spots for such antibiotic-resistant bacteria and their genes spread into the environment (Woerther *et al*., 2013). Methods to reduce the load of resistant bacteria

in the environment and the antimicrobial agents in the farms, include optimization of disinfection procedures and management of wastewater and manure. Limitations of the study were that MDR coding genes were not identified. There would have been recall bias by the participants on antibiotic usage and details of hospital visits. The environmental association with MDRO isolation was not studied.

Conclusion

This study provides important insights into the phenomenon of rising resistance in the community. While colonization of the gastrointestinal tract by MDROs, ESBL and AmpC *β*-lactamase producing enteric pathogen increases the risk of infection, there is also a possibility that mobile carriers' resistance genes will be spread into a broad community. Thus, there remains a great importance on proper detection and control of these isolates in the community. Strict regulations and monitoring over prescription & sale of antibiotics needs utmost attention to prevent the spread of drug resistance.

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Conflicts of interest:

There are no conflicts of interest.

References

Ahmed, Salwa F, Mostafa Mohamed M, Ali, Zienat K. Mohamed, Tarek A. Moussa, and John Klena D. 2014. "Fecal Carriage of Extended-Spectrum β-Lactamases and AmpC-Producing Escherichia Coli in a Libyan Community." Annals of Clinical Microbiology and Antimicrobials **13(1).**

Al-Agamy, Mohamed H, Taghrid S, El Mahdy and Atef Shibl M. 2016. "Fecal Colonization with Extended-Spectrum Beta-Lactamase and AmpC-Producing Escherichia Coli." BioMed Research International 2016.

Antony, Sherly, Kandasamy Ravichandran, and Reba Kanungo. 2018. "Multidrug-Resistant Enterobacteriaceae Colonising the Gut of Adult Rural Population in South India." Indian Journal of Medical Microbiology **36(4),** 488-93. https://doi.org/10.4103/ijmm.IJMM_18_388.

Baljin, Bayaraa, Ganbaatar Baldan, Battogtokh Chimeddorj, Khosbayar Tulgaa, Batbaatar Gunchin, Tsogtsaikhan Sandag, Klaus Pfeffer, Colin R. MacKenzie, and Andreas, Wendel F. 2016. "Faecal Carriage of Gram-Negative Multidrug-Resistant Bacteria among Patients Hospitalized in Two Centres in Ulaanbaatar, Mongolia." PLoS ONE **11(12).** https://doi.org/10.1371/journal.pone.0168146.

Casals-Pascual, Climent, Andrea Vergara, and Jordi Vila. 2018. "Intestinal Microbiota and Antibiotic Resistance: Perspectives and Solutions." Human Microbiome Journal **9,** 11-15. https://doi.org/10.1016/j.humic.2018.05.002.

Farra A, Frank T, Tondeur L, Bata P, Gody JC, Onambele M, Rafaï C, Vray M, Breurec S. 2016. "High Rate of Faecal Carriage of Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Healthy Children in Bangui, Central African Republic." Clinical Microbiology and Infection **22(10),** 891.e1-891.e4. https://doi. org/10.1016/j.cmi.2016.07.001.

Gargiullo, Livia, Federica Del Chierico, Patrizia D'Argenio, and Lorenza Putignani. 2019. "Gut Microbiota Modulation for Multidrug-Resistant Organism Decolonization: Present and Future Perspectives." *Frontiers in Microbiology*. Frontiers Media S.A. https://doi.org/10.3389/ fmicb.2019.01704.

Jakobsson, Hedvig E., Cecilia Jernberg, Anders F. Andersson, Maria Sjölund-Karlsson, Janet K. Jansson, and Lars Engstrand. 2010. "Short-Term Antibiotic Treatment Has Differing Long- Term Impacts on the Human Throat and Gut Microbiome." PLoS ONE **5(3).**

Karanika, Styliani, Theodoros Karantanos, Marios Arvanitis, Christos Grigoras, and Eleftherios Mylonakis. 2016. "Fecal Colonization with Extended-Spectrum Beta-Lactamase-Producing Enterobacteriaceae and Risk Factors among Healthy Individuals: A Systematic Review and Metaanalysis." Clinical Infectious Diseases **63(3),** 310-18. https://doi.org/10.1093/cid/ciw283.

Lautenbach, Ebbing, Jean Baldus Patel, Warren B. Bilker, Paul H. Edelstein, and Neil Fishman O. 2001. "Extended-Spectrum β-Lactamase-Producing Escherichia Coli and Klebsiella Pneumoniae: Risk Factors for Infection and Impact of Resistance on Outcomes." Clinical Infectious Diseases **32(8),** 1162-71. https://doi.org/10.1086/319757.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S. 2012. "Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance." Clinical Microbiology and Infection **18(3),** 268-81.

Mandal, Dipendra Kumar, Shiv Kumar Sah, Shyam Kumar Mishra, Sangita Sharma, Hari Prasad Kattel, Sanjeet Pandit, Pranav Kumar Yadav. 2020. "Carriage of Extended-Spectrum- β -Lactamase- And AmpC- β -Lactamase-Producing Enterobacteriaceae (ESBL-PE) in Healthy Community and Outpatient Department (OPD) Patients in Nepal." Canadian Journal of Infectious Diseases and Medical Microbiology 2020. https://doi.org/10.1155/2020/5154217.

Molton, James S, Paul A, Tambyah, Brenda SP, Ang, Moi Lin Ling, and Dale Fisher A. 2013. "The Global Spread of Healthcare-Associated Multidrug-Resistant Bacteria: A Perspective from Asia." Clinical Infectious Diseases **56(9),** 1310-18.

Monira, Shirajum, Syeda Antara Shabnam, Sk Imran Ali, Abdus Sadique, Fatema Tuz Johura, Kazi Zillur Rahman, Nur Haque Alam, Haruo Watanabe, and Munirul Alam. 2017. "Multi-Drug Resistant Pathogenic Bacteria in the Gut of Young Children in Bangladesh." Gut Pathogens **9(1).** https://doi.org/10.1186/s13099

Ojer-Usoz, Elena, David González, Ana Isabel Vitas, José Leiva, Isabel García-Jalón, Alejandro Febles-Casquero, and María de la Soledad Escolano. 2013. "Prevalence of Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae in Meat Products Sold in Navarra, Spain." Meat Science **93(2),** 316-21.

Pathak, Ashish, Salesh P. Chandran, Kalpana Mahadik, Ragini Macaden and Cecilia Stålsby Lundborg. 2013. "Frequency and Factors Associated with Carriage of Multi-Drug Resistant Commensal Escherichia Coli among Women Attending Antenatal Clinics in Central India." BMC Infectious Diseases **13(1).**

Sandhu, Raminder, Aditi Aggarwal, Pallavi Sayal and Surinder Kumar. 2019. "Intestinal Carriage of Drug-resistant Gram-negative Bacteria Belonging to Family Enterobacteriaceae in Children Aged 3–14 Years: An Emerging Threat." International Journal of Health & Allied Sciences **8,** 108-15.

Sayed, Amal Mohamed, Iman Kamal Behiry, Rasha Hamed Elsherief, and Sara Ali Elsir. 2017. "Detection of Carbapenemase-Producing Enterobacteriaceae in Rectal Surveillance Cultures in Non-Hospitalized Patients." Journal of Analytical Science and Technology **8(1).**

Shakya, Pragya, Peter Barrett, Vishal Diwan, Yogyata Marothi, Harshada Shah, Neeraj Chhari, Ashok J. Tamhankar, Ashish Pathak and Cecilia S. Lundborg. 2013. "Antibiotic Resistance among Escherichia Coli Isolates from Stool Samples of Children Aged 3 to 14 Years from Ujjain, India." BMC Infectious Diseases **13(1).**

Subramanya, Supram Hosuru, Indira Bairy, Niranjan Nayak, Shashiraja Padukone, Brijesh Sathian and Shishir Gokhale. 2019. "Low Rate of Gut Colonization by Extended-Spectrum β-Lactamase Producing Enterobacteriaceae in HIV Infected Persons as Compared to Healthy Individuals in Nepal." PLoS ONE **14(2).**

Valverde, Aránzazu, Fabio Grill, Teresa M. Coque, Vicente Pintado, Fernando Baquero, Rafael Cantón and Javier Cobo. 2008. "High Rate of Intestinal Colonization with Extended-Spectrum-β-Lactamase-Producing Organisms in Household Contacts of Infected Community Patients." Journal of Clinical Microbiology **46(8),** 2796-99.

Wayne PA. 2011. "Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. www.sid.ir/en/Journal/ViewPaper.aspx?ID=450165.

Woerther, Paul Louis, Charles Burdet, Elisabeth Chachaty and Antoine Andremont. 2013. "Trends in Human Fecal Carriage of Extended-Spectrum β-Lactamases in the Community: Toward the Globalization of CTX-M." *Clinical* Microbiology Reviews **26(4),** 744-58. https://doi.org/10.1128/CMR.00023-13.