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Prevalence of *Escherichia coli* Resistant to Beta-Lactam Antibiotics among Patients with Chronic Obstructive Pulmonary Disease and Urinary Tract Infection

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Urinary tract infection (UTI), which is typically caused by *Escherichia coli* (*E. coli*), is an insufficiently recognized co-morbidity among patients with chronic obstructive pulmonary disease (COPD). Adequate treatment can be complicated by resistance of the causative pathogen to beta-lactam antibiotics, which often produce beta-lactamase enzymes that destroy the antibiotic. The beta-lactamase family of enzymes is extremely diverse, including different types of enzyme and mutant forms. In this study, we analyzed 580 patients with COPD (236 females and 344 males) and thus found 218 patients with co-morbid UTIs, including 58 patients with UTI caused by *E. coli* (38 females and 20 males). We also investigated cases of uncomplicated symptomatic and asymptomatic UTI caused by *E. coli* and the presence of resistance to beta-lactam antibiotics among those patients. The *E. coli* strains resistant to beta-lactam antibiotics were selected for their ability to grow on selective media, before DNA microarrays were applied for specific identification of three beta-lactamase gene types (i.e., TEM, SHV and CTX-M). Overall, 83% of *E. coli* strains responsible for UTIs in COPD patients carried extended-spectrum beta-lactamase genes. The most prevalent were those producing CTX-M, with CTX-M-15 being predominant. The rare CTX-M-27 and TEM-15 genes were also detected in two samples. Three samples contained several extended-spectrum beta-lactamase genes simultaneously (CTX-M-15 or CTX-M-14 plus SHV-5 or TEM-15). This high prevalence of resistant *E. coli* strains necessitates rational antibiotic selection when treating UTI to prevent COPD exacerbations. Additionally, antibiotic therapy should be aligned with and adapted to existing and potential COPD co-morbidities.

Keywords: antibiotic resistance; beta-lactamases; chronic obstructive pulmonary disease; *Escherichia coli*; urinary tract infection
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Introduction

Among patients with chronic obstructive pulmonary disease (COPD), the presence of chronic inflammation leads to a strong interaction with co-morbidities (Barnes and Celli 2009). The most common of these are lung carcinoma, loss of skeletal musculature, cachexia (loss of fat), pulmonary hypertension, ischaemic heart disease, hyperlipidaemia, congestive heart failure, normocytic anaemia, diabetes, metabolic syndrome, osteoporosis, obstructive sleep apnoea, depression and arthritis (Mannino et al. 2015). Given the additional association of COPD with increased age, it is not surprising that quality of life can be significantly decreased. However, urinary tract infection (UTI) is another co-morbidity that is insufficiently recognised.

UTIs affect people of all age groups, but particularly children, young women and the elderly. Notably, approximately 40% of women are expected to experience more than one UTI in their lifetimes (Flores-Mireles et al. 2015). These infections are traditionally classified according to clinical symptoms, laboratory findings and microbiological analyses as cystitis (infections of the lower urinary tract or bladder), pyelonephritis (infections affecting the upper urinary tract or the kidneys) and prostatitis. Prior to this, UTIs were classified by their clinical features and association with treatment and morbidity. This earlier categorisation included non-complicated cystitis in younger females, recurrent cystitis in younger females, acute uncomplicated pyelonephritis in younger females, complicated UTIs, UTIs related to indwelling catheters, UTIs in men and asymptomatic bacteriuria (Martin 2013).

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Acute complicated urinary infections caused by Gram-negative bacteria are rare, but they can cause septic shock; periurethral abscess; renal and perinephric abscess; and metastatic infections of the bones, joints and heart (endocarditis). These complications mostly develop in patients with co-morbidities (e.g., diabetes mellitus), chronic urological devices, urinary obstructions or renal failure (Sherchan et al. 2015). Among the Gram-negative bacteria known to cause symptomatic and asymptomatic UTIs, *Escherichia coli* is particularly common among females (80-85%) compared with males (George et al. 2015).

Systemic antibiotics are crucial for the treatment of severe and life-threatening infective exacerbations of COPD. However, the presence of concomitant infections complicates the choice of therapy, especially given that *E. coli* strains resistant to beta-lactam antibiotics developed during therapy. Consequently, hidden or active UTI can arise during the stable disease phase of COPD. Beta-lactam antibiotics (i.e., penicillins, cephalosporins, monobactams and carbapenems) are the most commonly used treatments for infections caused by Gram-negative bacteria, but their effectiveness has reduced significantly because of the spread of resistant strains (Bush 2013). The most prevalent mechanism of the resistance is the production of beta-lactamases that hydrolyse the drugs (George et al. 2015). Beta-lactamases are a diverse class of enzymes that alter the structure and profile of antibiotics (Poirel et al. 2012). Currently, extended-spectrum beta-lactamases (ESBLs), which hydrolyse penicillins and cephalosporins, pose significant danger to clinical practice. The most prevalent among these are the serine beta-lactamases (*bla* gene) of molecular class A, which include types CTX-M, SHV and TEM (Poirel et al. 2012).

Although cephalosporins and carbapenems are otherwise the most effective agents for inhibiting Gram-negative bacilli, decreased bacterial sensitivity means that their use must be strictly controlled. Quinolones can also be used for the empirical treatment of pneumonia and infective exacerbations of COPD, without the risk of promoting the development of antibiotic-resistant bacteria (Fumagalli et al. 2015). In the future, inhaled antibiotics may provide a more targeted approach for the prevention and treatment of infections in COPD (Fumagalli et al. 2015). Overall, there is a need to estimate the potential long-term effects of systemic antibiotics use in patients treated for COPD or for UTIs in the context of co-morbid COPD and how this related to the development of bacterial resistance.

In this study, we aimed to determine: 1) the presence of a UTI with significant bacteriuria, asymptomatic bacteriuria and *E. coli* in the urinary tract infection; 2) the resistance of *E. coli* to penicillin and cephalosporin; and 3) beta-lactamase genes in genetic structure of *E. coli* among COPD patients.

Methods

This was a case-control study of adults with COPD and co-morbid UTI caused by *E. coli*. Urine samples were obtained from 580 patients treated for COPD at the Mediterranean health centre Igalo between January 2016 and June 2017. COPD was diagnosed and staged according to the (GOLD, Global Initiative for Chronic Obstructive Lung Disease, 2017) criteria. Patients were excluded if they had treatment-related UTI, COPD with complications or were smokers. The study was conducted in accordance with the ethical standards of the Committee on Human Experimentation as set by the Physiotherapists' Association of Montenegro (No. 01-28/08-3/2017) in accordance with the Declaration of Helsinki. All individuals provided signed informed consent to participate in the study.

Urine was analysed 1 day after consultation with a doctor. If leukocyte esterase or nitrites were present on dipstick test, along with pyuria (> 10 leukocytes/high-powered field), the sample was centrifuged and observed microscopically. Significant bacteriuria was defined as samples with > 100,000 colony-forming units per millilitre. The procedure was repeated after 1 month in patients with COPD who had a confirmed UTI.

UTI was defined as the presence of urinary symptoms with significant bacteriuria after urine culture. Asymptomatic bacteriuria was defined as the presence of significant bacteriuria in two urine cultures after 7 days in patients with no symptoms. Pyelonephritis was defined by the presence of urinary symptoms, significant bacteriuria, pyrexia, abdominal pain and increasing C-reactive protein and leukocyte levels. Repeat UTIs were considered as two or more in the previous year.

All urine samples were investigated at the microbiological laboratory of Institute for Public Health of Montenegro and the microbiological laboratory of the Health Centre in Herceg Novi. The following were tested: significant or asymptomatic bacteriuria; *E. coli* as the UTI-causing pathogen; antibiotic resistance (by phenotyping); and DNA genotyping of isolated *E. coli* strains for the presence of *bla* genes of molecular class A (TEM, SHV and CTX-M types). During laboratory testing, urine cultures were performed, and those positive for *E. coli* were collected and tested using a BD BBL™ CHROMagar™ ESBL bi-plate with a selective homogenous plate to screen for Gram-negative bacilli and ESBLs. Plasmid DNA was then isolated from bacterial cultures and used as a template for multiplex polymerase chain reaction (PCR) with six pairs of primers specific for beta-lactamase genes. Amplification was performed in a Master cycler gradient thermal cycler (Eppendorf AG, Hamburg, Germany). An initial denaturation step (94°C for 2 min) was followed by 30 cycles (94°C for 20 s, 60°C for 30 s and 72°C for 45 s) and a final extension step at 72°C for 4 min. PCR products were analysed by electrophoresis on 1.5% agarose gel.

The biotin-labelled amplicons were used as target DNA for genotyping on colorimetric DNA microarrays (Rubtsova et al. 2010). This technique presents a genotyping platform that allows the analysis of many genetic determinants in a single assay. The identification principle was based on hybridisation of a target DNA labelled with biotin with different specific oligonucleotide probes immobilised on a small microchip (1.5 cm^2). The probe sequence corresponded to conserved fragments of the different *bla* genes. Identification of genes on the microarray consisted of four steps: (1) isolation of bacterial DNA from a clinical sample, (2) amplification of the *bla* gene in a multiplex PCR with simultaneous labelling, (3)

hybridisation of a target DNA with oligonucleotide probes on the surface of the microarray and (4) detection of hybridisation results. Because of the hybridisation reaction, biotin-labelled DNA duplexes were formed on the surface of the microarray. Biotin was detected using the conjugate of streptavidin and horseradish peroxidase. Peroxidase was then detected colorimetrically by accumulation of the insoluble coloured product of the enzymatic reaction. As a result, coloured spots were formed in the microarray zones in which hybridisation occurred. The intensity of the staining, if significantly different from the background signal, indicated the presence of a gene with a sequence complementary to the oligonucleotide probe.

Results

Urine samples were obtained from 580 patients (344 males and 236 females) treated for COPD during the study period. The basic characteristics of those patients are shown in Table 1. The participants were obese on the basis of their body mass index, and most had a COPD stage of I (61%; n = 354) or II (20%; n = 116). Moreover, 218 patients (37%) had co-morbid UTIs (61% were female, and 39% were male), and most of these occurred in patients with COPD of stages II and III (50% and 24%, respectively). Only 58 patients (10%) suffered from UTI caused by *E. coli*, and most of these were in patients with COPD of stages II and III (54% and 20%, respectively). Table 2 shows that significant bacteriuria, asymptomatic bacteriuria, cystitis, pyelonephritis and recurrent UTI were present in 97%, 3%, 94%, 1% and 5%, respectively.

Urine cultures positive for *E. coli* were collected and tested to identify resistance to beta-lactam antibiotics, using DNA microarray for selective identification of beta-lactamases. In total, the microarray contained 75 oligonucleotide probes to identify TEM, STV and CTX-M beta-lactamases and polymorphisms responsible for ESBL phenotypes (i.e., those with resistance to penicillins and first- to fourth-generation cephalosporins).

Plasmid DNA was isolated from 47 *E. coli* cultures and used as a template for multiplex PCR, which resulted in sufficient numbers of DNA amplicons being collected from 40 samples (85.1%). As shown in Table 3, ESBL genes

Table 1. Basic characteristics of patients with COPD.

Characteristic	Total	UTI	UTI caused by *E. coli*
Number of patients	580 (100%)	218 (37%)	58 (10%)
Average age	56.3 ± 1.7 years	60 ± 1.0 years	61 ± 0.1 years
Female	236 (40%)	133 (61%)	38 (66%)
Male	344 (60%)	85 (39%)	20 (34%)
Average BMI	33 ± 1.0 kg/m^2	31 ± 1.0 kg/m^2	31 ± 1.0 kg/m^2
COPD stage I	354 (61%)	30 (14%)	8 (14%)
COPD stage II	116 (20%)	109 (50%)	31 (54%)
COPD stage III	64 (11%)	52 (24%)	12 (20%)
COPD stage IV	46 (8%)	27 (12%)	7 (12%)

BMI, body mass index; COPD, chronic obstructive pulmonary disease; *E.coli*, *Escherichia coli*; UTI, urinary tract infection.
(January 1, 2016 to June 3, 2017; Mediterranean health centre Igalo.)

Table 2. Distribution of UTI category and bacteriuria type by sex.

	Cystitis	**Pyelonephritis**	**RU**	**SB**	**AB**
Total Patients	204 (94%)	3 (1%)	11 (5%)	203 (97%)	15 (3%)
Female	122 (60%)	3 (100%)	8 (72%)	123 (61%)	10 (67%)
Male	82 (40%)	-	3 (28%)	80 (39%)	5 (33%)

AB, asymptomatic bacteriuria; RU, recurrent UTI; SB, significant bacteriuria; UTI, urinary tract infection.
(January 1, 2016 to June 3, 2017; Mediterranean health centre Igalo.)

Table 3. Beta-lactamase genes detected in *Escherichia coli* strains by hybridisation on DNA microarray.

Plasmid DNA samples	Number of samples, $n = 47$ (100%)	Beta-lactamase genes detected	ESBL	Resistant to penicillins and cephalosporins
L11, L30, L70, L71, L78, L88, L96	7 (14.9%)	No genes detected	No genes detected	-
L17	1 (2.1%)	TEM-1	No genes detected	Generation I
L6, L63, L75	3 (6.4%)	CTX-M-15	CTX-M-15	Generations I-IV
L76	1 (2.1%)	CTX-M-27	CTX-M-27	Generations I-IV
L1–L5, L7, L12, L13, L18, L21–L23, L27, L34, L38, L43, L46, L48–L50, L57, L58, L72, L77, L87, L89, L95	27 (57.4%)	TEM-1, CTX-M-15	CTX-M-15	Generations I-IV
L33	1 (2.1%)	TEM-1, CTX-M-3	CTX-M-3	Generations I-IV
L24, L29	2 (4.3%)	TEM-1, CTX-M-9/14	CTX-M-9/14	Generations I-IV
L16, L44	2 (4.3%)	TEM-1, SHV-1 CTX-M-15	CTX-M-15	Generations I-IV
L97	1 (2.1%)	TEM-15, CTX-M-15	TEM-15, CTX-M-15	Generations I-IV
L35, L67	2 (4.3%)	TEM-1, SHV-5, CTX-M-14	SHV-5, CTX-M-14	Generations I-IV

ESBL, extended spectrum of beta-lactamases genes; TEM, SHV, CTX, beta-lactamase genes.

were present in 39 of the 47 *E. coli* samples (83%), with the beta-lactamase CTX-M types present in all ESBL-positive cultures. Three samples contained several ESBL genes simultaneously (CTX-M-15 and TEM-15; CTX-M-14 and SHV-5). The most dominant was CTX-M-15. Samples L76 and L97 contained CTX-M-27 and a mixture of TEM-15 and CTX-M-15, respectively. That was considered interesting and specific in our area of research.

Discussion

COPD is a complex disease that manifests with both pulmonary and extrapulmonary symptoms. Moreover, in excess of 30% of patients have one additional chronic disease and 40% have two or more co-morbidities, but only some of these are recognised by GOLD (2017). Clinically, co-morbidities not only prolong hospitalisations but are also a key measure of disease progression. Repeated co-morbidities reflect the progression of chronic systemic inflammation and oxidative stress that cause body temperature dysfunction and DNA damage (Dal Negro et al. 2015). However, many researchers believe that some valid COPD-related co-morbidities remain unrecognised, with symptoms being neglected or difficult to differentiate from symptoms of complications or side effects of therapy (Corlateanu et al. 2016).

Previous researchers have argued that UTIs are not a true co-morbidity in patients treated for COPD. Among the prevailing urogenital co-morbidities associated with COPD, chronic kidney disease (15%) and benign prostatic hyperplasia (7-10%) are noted as the main conditions (Miłkowska-Dymanowska et al. 2015). Additionally, urologists have reported that bronchial asthma is a co-morbidity in 8% of urinary tract diseases (Aboumarzouk 2014). In the present research, we additionally showed that UTI infections were present in 218 patients (37%), whereas significant bacteriuria and asymptomatic bacteriuria were noted in 203 (97%) and 15 (3%) patients, respectively. Among the cases of UTI, cystitis dominated (94%; $n = 204$), with most cases in females.

Most epidemiologic studies of UTI have identified *E. coli* as the main infectious pathogen among the female population, irrespective of age. In 1980, non-chromosomal, plasmid-encoded ampC was discovered in *Enterobacteriaceae* and was thought to have developed because of the common

use of cephalosporins (Auer et al. 2010). This bacterial beta-lactamase possesses the capacity to inactivate practically all cephalosporins (Simon et al. 2010). Additionally, multi-resistant isolates that produce ESBLs have spread. They are often isolated in UTIs and are typically resistant to quinolones, aminoglycosides, sulfonamides, ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole (Hooton 2012).

The spread of Gram-negative bacteria bearing plasmid-encoded ESBLs is recognised as a worldwide problem. The prevalence of these bacteria is increased in both hospital-acquired and environmental infections. Despite the treatment, the municipal sewage may be a reservoir of antibiotic-resistance genes. This may pose a public health risk, which requires future evaluation and control (Korzeniewska and Harnisz 2013).

In the current research, urine culture of patients showed that *E. coli* was responsible for 85.5% of UTIs in patients with grades II and III COPD. Of these, 83% were positive for ESBLs and produced CTX-M beta-lactamases, with the most dominant being CTX-M-15. Some of the samples carried several beta-lactamase genes, including a mixture of one ESBL and penicillinase TEM-1 or a mixture of two ESBLs and SHV-1. It was interesting that two rare variants, CTX-M-27 and TEM-15, were found specific to our area. These are important because beta-lactamase CTX-M-27, which was first isolated in France from a clinical strain of *E. coli* in 2000 (Bonnet at al. 2003), has the key mutation D240G. Beta-lactamase CTX-M-27 was the third CTX-M enzyme shown to harbour this mutation after CTX-M-15 and CTX-M-16, with resulting enzymes having higher minimum inhibitory concentrations to ceftazidime.

It appears the existence of discrepancy in resistance and sensitivity to ESBL organisms by geographical location (Picozzi et al. 2013). Snapshot of highly virulent/ multiresistant clone ST131 of uropathogenic *E. coli* was reported from India (Hussain et al. 2012).

European guidance for the treatment of these resistant infections recommends using aminoglycosides and carbapenems as first-line therapy. Every region or area of Europe should investigate which bacteria is dominant and then check its sensitivity to antibiotics. Such an approach reduces the number of complicated infections. In cases of complicated UTI, where there is a high risk for the presence of ESBL infection, it is important to test urine microscopy, culture and sensitivity without delay. At the same time, however, doctors should consider the risk factors for ESBL UTIs, symptomatic bacteriuria, asymptomatic bacteriuria and complicated infections (Briongos-Figuero et al. 2012).

Analysis of the treated COPD population by disease severity indicated that increasing the severity of COPD increased the likelihood of UTIs and beta-lactamase-producing *E. coli*. Previous studies have indicated that disease progression when using additional drugs, especially corticosteroids, can lead to immunity problems and good conditions for the development of UTIs (Peirano et al. 2012). Unfortunately, no association has been found between inhaled long-acting anticholinergics and various measures of UTI in older patients with COPD. It has also been shown that there is no increased risk of UTI in older males, or indeed females (Gershon et al. 2017), with newly diagnosed COPD.

Among our COPD groups, in which the mean age was 56 ± 1.7 years, the average ages of those with UTIs and UTIs caused by *E. coli* were 60 ± 1.0 and 61 years, respectively. Thus, older patients may require stricter control of COPD and urine screening for ESBL-producing strains. Additionally, the high prevalence of UTI among elderly women was confirmed (Marques et al. 2012). Regardless, antibiotics must be carefully selected and adapted to the patients' needs, and dosages must be appropriate to kidney function (Barakat et al. 2015).

UTIs also remain a complex problem in terms of diagnosis, because patients often have non-specific symptoms. The most common symptom is change in the smell of urine (60.6%), and predisposing factors for UTIs include repeated UTIs, vaginitis and diabetes (Zalmanovici et al. 2015). Each of these should be considered in any patient assessment, but especially in older patients. In the current research, we only identified 15 cases (3%) of asymptomatic bacteriuria in patients with COPD. When symptoms are not recognisable, treatment delays and antibiotic effects are trivial (Dull et al. 2014). Researchers have therefore asserted that doctors should be aware of the typical symptoms required to diagnose UTI.

In this study, 40 patients (85%) developed resistance to cephalosporins, which was proven by microbiological analysis of all 47 samples containing *E. coli*. Antimicrobial resistance is related to a given community because of local prescribing practices (Miyoshi-Akiyama et al. 2016). Population ageing worldwide has also been associated with an increase in the number of infectious diseases, with immunodeficiency being dependent not only on age but also on hormonal changes, poor nutrition and diabetes (Mody and Juthani-Mehta 2014). These diseases are among the leading co-morbidities in COPD.

In conclusion, quality of life in older patients with stage III or IV COPD could be improved by providing detailed urinalysis. Besides investigating bacterial culture, there is a need to test for beta-lactam resistance in isolated soil bacteria. Microbiological analysis of ESBL-positive E. coli in the urine has shown the genetic specificity of CTX-M-27, CTX-M-14 and SHV-5. Moving forward, we must ensure adequate knowledge about the etiology of these diseases and improve the management of COPD and UTI to decrease the incidence of resistance to new antibiotic generations and reduce the costs of health protection. Rational antibiotic use in patients with COPD who develop UTI should be based on the following: (1) knowledge of resistant species associated with UTI; (2) the antibiotics used in previous months; and (3) microbial resistance levels in the local community; and (4) the empirical antibiotic

therapy used before urine culture results were available. Despite these conclusions, it should be noted that this research was limited by a small sample size and by failure to repeat the microbiological analyses for samples with low DNA concentrations. Consequently, we yielded some uncertain results that require checking in further study.

Conflict of Interest

The authors declare no conflict of interest.

References

Aboumarzouk, O.M. (2014) Extended spectrum beta-lactamase urinary tract infections. *Urol. Ann.*, **6**, 114-115.

Auer, S., Wojna, A. & Hell, M. (2010) Oral treatment options for ambulatory patients with urinary tract infections caused by extended-spectrum-*β*-lactamase-producing *Escherichia coli*. *Antimicrob. Agents Chemother.*, **54**, 4006-4008.

Barakat, M.F., McDonald, H.I., Collier, T.J., Smeeth, L., Nitsch, D. & Quint, J.K. (2015) Acute kidney injury in stable COPD and at exacerbation. *Int. J. Chron. Obstruct. Pulmon. Dis.*, **10**, 2067-2077.

Barnes, P.J. & Celli, B.R. (2009) Systemic manifestations and comorbidities of COPD. *Eur. Respir. J.*, **33**, 1165-1185.

Bonnet, R., Recule, C., Baraduc, R., Chanal, C., Sirot, D., De Champs, C. & Sirot, J. (2003) Effect of D240G substitution in a novel ESBL CTX-M-27. *J. Antimicrob. Chemother.*, **52**, 29-35.

Briongos-Figuero, L.S., Gómez-Traveso, T., Bachiller-Luque, P., Domínguez-Gil González, M., Gómez-Nieto, A. Palacios-Marin, T., González-Sagrado, M., Dueñas-Laita, A. & Pérez-Castrillón, J.L. (2012) Epidemiology, risk factors and comorbidity for urinary tract infections caused by extended-spectrum beta-lactamase (ESBL)-producing enterobacteria. *Int. J. Clin. Pract.*, **66**, 891-896.

Bush, K. (2013) Proliferation and significance of clinically relevant *β*-lactamases. *Ann. NY Acad. Sci.*, **1277**, 84-90.

Corlateanu, A., Covantev, S., Mathioudakis, A.G., Botnaru, V. & Siafakas, N. (2016) Prevalence and burden of comorbidities in chronic obstructive pulmonary disease. *Respir. Investig.*, **54**, 387-396.

Dal Negro, R.W., Bonadiman, L. & Turco, P. (2015) Prevalence of different comorbidities in COPD patients by gender and GOLD stage. *Multidiscip. Respir. Med.*, **10**, 24.

Dull, R.B., Friedman, S.K., Risoldi, Z.M., Rice, E.C., Starlin, R.C. & Destache, C.J. (2014) Antimicrobial treatment of asymptomatic bacteriuria in non-catheterized adults. *Pharmacotherapy*, **34**, 941-960.

Flores-Mireles, A.L., Walker, J.N., Caparon, M. & Hultgren, S.J. (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. *Nat. Rev. Microbiol.*, **13**, 269-284.

Fumagalli, G., Fabiani, F., Forte, S., Napolitano, M., Balzano, G., Bonini, M., De Simone, G., Fuschillo, S., Pentassuglia, A., Pasqua, F., Alimonti, P., Carlone, S. & Sanguinetti, C.M. (2015) INDACO project: COPD and link between comorbidities, lung function and inhalation therapy. *Multidiscip. Respir. Med.*, **10**, 4.

George, C.E., Norman, G., Ramana, G.V., Mukherjee, D. & Rao, T. (2015) Treatment of uncomplicated symptomatic urinary tract infections: resistance patterns and misuse of antibiotics. *J. Family Med. Prim. Care*, **4**, 416-421.

Gershon, A.S., Newman, A.M., Fischer, H.D., Austin, P.C., Daneman, N., Bell, C.M., Stephenson, A.L., Gill, S.S., Vozoris, N.T. & Rochon, P.A. (2017) Inhaled long-acting anticholinergics and urinary tract infection in individuals with COPD. *COPD*, **14**,105-112.

Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2017) Global strategy for the diagnosis, management and prevention of COPD; 2017. http://goldcopd.org/gold-2017-global-strategy-diagnosis-management-prevention-copd/ [*Accessed*: February 15, 2017].

Hooton, T.M. (2012) Clinical practice. Uncomplicated urinary tract infection. *N. Engl. J. Med.*, **366**, 1028-1047.

Hussain, A., Ewers, C., Nandanwar, N., Guenther, S., Jadhav, S., Wieler, L.H. & Ahmed, N. (2012) Multiresistant uropathogenic *Escherichia coli* from a region in India where urinary tract infections are endemic: genotypic and phenotypic characteristics of sequence type 131 isolates of the CTX-M-15 extended-spectrum-*β*-lactamase-producing lineage. *Antimicrob. Agents Chemother.*, **56**, 6358-6365.

Korzeniewska, E. & Harnisz, M. (2013) Extended-spectrum beta-lactamase (ESBL)-positive enterobacteriaceae in municipal sewage and their emission to the environment. *J. Environ. Manage.*, **128**, 904-911.

Mannino, D.M., Higuchi, K., Yu, T.C., Zhou, H., Li, Y., Tian, H. & Suh, K. (2015) Economic burden of COPD in the presence of comorbidities. *Chest*, **148**, 138-150.

Marques, L.P., Flores, J.T., De Oliveira Barros, O. Jr., Rodrigues, G.B., de Medeiros Mourão, C. & Moreira, R.M. (2012) Epidemiological and clinical aspects of urinary tract infection in community-dwelling elderly women. *Braz. J. Infect. Dis.*, **5**, 436-441

Martin, K.E. (2013) Treatment of ESBL-producing *E. coli* lower urinary tract infection. http://www.pharmacytimes.com/publications/health-system-edition/2013/november2013/treatment-of-esbl-producing-e-coli-lower-urinary-tract-infection [*Accessed*: April 10, 2017].

Miłkowska-Dymanowska, J., Białas, A.J., Zalewska-Janowska, A., Górski, P. & Piotrowski, W.J. (2015) Underrecognized comorbidities of chronic obstructive pulmonary disease. *Int. J. Chron. Obstruct. Pulmon. Dis.*, **10**, 1331-1341.

Miyoshi-Akiyama, T., Sherchan, J.B., Doi, Y., Nagamatsu, M., Sherchand, J.B., Tandukar, S., Ohmagari, N., Kirikae, T., Ohara, H. & Hayakawa, K. (2016) Comparative genome analysis of extended-spectrum-beta-lactamase-producing *Escherichia coli* sequence type 131 strains from Nepal and Japan. *mSphere*, **1**, e00289-16.

Mody, L. & Juthani-Mehta, M. (2014) Urinary tract infections in older women. *JAMA*, **311**, 844-854.

Peirano, G., van der Bij, A.K., Gregson, D.B. & Pitout, J.D. (2012) Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum *β*-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. *J. Clin. Microbiol.*, **50**, 294-299.

Picozzi, S., Ricci, C., Gaeta, M., Macchi, A., Dinang, E., Paola, G., Tejad, M., Costa, E., Bozzini, G., Casellato, S. & Carmignani, L. (2013) Do we really know the prevalence of multi-drug resistant *Escherichia coli* in the territorial and nosocomial population? *Urol. Ann.*, **5**, 25-29.

Poirel, L., Bonnin, R.A. & Nordmann, P. (2012) Genetic support and diversity of acquired extended-spectrum *β*-lactamases in Gram-negative rods. *Infect. Genet. Evol.*, **12**, 883-893.

Rubtsova, M.Y., Ulyashova, M.M., Edelstein, M.V. & Egorov, A.M. (2010) Oligonucleotide microarrays with horseradish peroxidase-based detection for the identification of extended-spectrum *β*-lactamases. *Biosens. Bioelectron.*, **26**, 1252-1260.

Sherchan, J.B., Hayakawa, K., Miyoshi-Akiyama, T., Ohmagari, N., Kirikae, T., Nagamatsu, M., Tojo, M., Ohara, H., Sherchand, J.B. & Tandukar, S. (2015) Clinical epidemiology and molecular analysis of extended-spectrum-*β*-lactamase-producing *Escherichia coli* in Nepal: characteristic of sequence types 131 and 648. *Antimicrob. Agents Chemother.*, **59**, 3424-3432.

Simon, A., Alexandra, W. & Markus, H. (2010) Oral treatment options for ambulatory patients with urinary tract infections caused by extended-spectrum-β-lactamase-producing *Escherichia coli*. *Antimicrob. Agents Chemother.*, **58**, 1252-1256.

Zalmanovici, T.A., Lador, A., Sauerbrun-Cutler, M. & Leibovici, L. (2015) Antibiotic treatment for asymptomatic bacteriuria. http://www.cochrane.org/CD009534/RENAL [*Accessed*: April 10, 2017].