



Burden of Gonorrhoea and Trends in Antibiotic Susceptibility Pattern of *Neisseria gonorrhoea* in Bhutan Over Four Years (2012-15)

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Authors' contributions

This work was a result of multi-site collaboration between all authors. Author TT conceived, designed and wrote the study protocol. Authors RS, NT, K. Wangchuk, TD, SW, VC, DD and K. Wangdi reviewed the proposal, participated in data collection and sought administrative approval at their respective hospitals. Authors TT and K. Wangdi analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Gonorrhoea is a worldwide public health problem. In Bhutan, the incidence of sexually transmitted infections increased from 12/10,000 in 2011 to 92/10,000 population in 2015. Disease burden and antibiotic resistance of *Neisseria gonorrhoea* have never been studied.

Aim: To study the burden of gonorrhoea and trends in antibiotic susceptibility pattern of *N. gonorrhoea* in Bhutan.

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Study Design: A descriptive, retrospective study.

Place of Study: The study was carried out in four large hospitals, the only hospitals with microbiology culture facilities in Bhutan; the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH), Eastern Regional Referral Hospital (ERRH), Central Regional Referral Hospital (CRRH), and Phuentsholing General Hospital (PGH).

Methods: Laboratory data of patients investigated for gonococcal urethritis in the four hospitals; over four years (2012-15) were analyzed. Laboratory processes involved microscopy by Gram stain and culture.

Results: A total of 1,858 samples were processed; JDWNRH (60.2%, 1,119), PGH (17.7%, 329), CRRH (11.5%, 214) and ERRH (10.6%, 196). Only 1.2% (22) was females. The commonest age group was 20-30 years. There was an increasing trend in the number of cases in all hospitals. Microscopically, Gram-negative diplococci were seen in 80.7% (1,499) of cases and *N. gonorrhoea* was isolated in 78.3% (1,173). Only 13.6% (3/22) were culture positive in females. Resistance against nalidixic acid, ciprofloxacin, penicillin, and tetracycline were >70% but against ceftriaxone (currently recommended antibiotic), azithromycin, spectinomycin and cefpodoxime were <5% (critical resistance level for recommended therapy).

Conclusion: The burden of gonorrhea increased over years but there were only a few female patients indicating poor adherence to 'seek and treat partners' advocated in treatment guidelines. Compliance with treatment guidelines warrants reinforcement. With ceftriaxone resistance of 0.2%, Bhutan can continue to use it as recommended therapy with ongoing, multi-site, resistance surveillance as resistances continue to emerge and spread worldwide. Such surveillance would provide early warning or evidence to base future changes in therapeutic choices.

Keywords: Antibiotic resistance; Bhutan; gonorrhoea; *Neisseria gonorrhoea*.

1. INTRODUCTION

Gonorrhoea is a sexually transmitted infection (STI) caused by the Gram-negative diplococcus, *Neisseria gonorrhoeae*. Gonorrhoea remains a significant public health problem worldwide. The global burden of gonorrhoea has increased by 21% in three years with a global incidence of 106.1 million cases in 2008 compared to 87.7 million cases in 2005 [1]. The global male to female ratio of new cases was reported to be 1.19 and 1.14 in 2005 and 2008 respectively [1]. Adding to the problem, gonococcal infections are becoming increasingly difficult to treat as *N. gonorrhoeae* evolves into a 'superbug' and may become untreatable as it becomes resistant to all classes of currently available antimicrobials [2]. The main prevention and control strategy for gonorrhoea is through identification and prompt treatment of cases. A successful, often single-dose, antibiotic treatment of gonococcal infection, administered at the time of diagnosis, is important for the cure of the individual patient, prevention of complications, and reduction of transmission [3]. Information on antimicrobial susceptibility of *N. gonorrhoeae* is important in the selection of an appropriate empirical antimicrobial agent. Surveillance of antimicrobial resistance in gonococci is aimed at detecting levels of resistance of ~5%, because, at this level of resistance, consideration should be given

to change the recommended treatment regimens [4]. Therefore, a surveillance program for antimicrobial resistance in *N. gonorrhoea* should ideally be comprehensive, active and ongoing [5]. Antibiotic resistance in *N. gonorrhoea* has followed the same general pattern for many decades, with the introduction of each new therapeutic agent followed by the development of resistance after a few years. Resistance to penicillin and tetracycline were seen in Asia as early as 1970 and to fluoroquinolones in the mid-1990s, both becoming widespread regionally and internationally. Resistance to ceftriaxone has now been reported from all regions of the World Health Organization (WHO) [6]. In Vietnam, resistance against ciprofloxacin was 98%, tetracycline 82%, penicillin G 48%, azithromycin 11%, ceftriaxone 5%, cefixime 1%, and spectinomycin 0% [7]. In the WHO southeast Asia region (SEAR), resistance to ceftriaxone was heterogeneous ranging from 18% in Myanmar (18 isolates) to 3.9% in India. However, there were no reports of ceftriaxone resistance in Nepal (7 isolates) and Sri Lanka (75 isolates). Resistance to ciprofloxacin increased from 9% in 1997 to 87% in 2006 in Bangladesh [8] and from 3.4% in 1996 to 83.3% in 2008 in India [9].

STIs remain a serious public health problem in Bhutan. The annual report compiled by the Bhutan Ministry of Health (MoH) revealed an

increase in the incidence of STIs (all STIs inclusive) from 12/10,000 population in 2011 to 92/10,000 population in 2015 [10]. However, no specific reports or studies on gonorrhoea have been undertaken to date. A study on STIs has shown variable understanding of STIs and their complications among the study participants. There was high sexual promiscuity in general and about a third, especially men engaged in risky sexual behaviors with low condom use [11]. Medical laboratory capacity is limited and apart from HIV, syphilis and gonorrhoea, no other bacterial or viral STIs can be routinely tested in Bhutan. Only four hospitals; the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH), Thimphu, Eastern Regional Referral Hospital (ERRH), Mongar, Central Regional Referral Hospital (CRRH), Gelephu and Phuentsholing General Hospital (PGH), Chukha, have microbiology culture facilities. Gonorrhoea is mostly managed syndromically based on clinical diagnosis. Bhutan has never conducted any antibiotic resistance surveillance to determine the local antibiotic resistance level to guide the development of standard treatment guidelines for gonorrhoea and national treatment guidelines have always been adapted from regional WHO guidelines until now. At present, gonorrhoea is treated with a single dose of ceftriaxone 250 mg by intramuscular injection [12]. Partner tracing and treatment is highly advocated in the STI treatment guideline but is rarely undertaken. A regional study with only seven *N. gonorrhoea* isolates from Bhutan revealed highest resistance level to ciprofloxacin (94%) followed by penicillin G (68%), erythromycin (62%), tetracycline (55%), azithromycin (7.7%) and no resistance to ceftriaxone, cefixime, and spectinomycin [13]. In 2008, 161 isolates of *N. gonorrhoea* showed 100% resistance to penicillin and 95% to quinolones in Bhutan [9]. A larger, more recent report showed the current resistance of *N. gonorrhoea* to ceftriaxone in Bhutan to be between 0.16-2.2% [6]. This is the first time a comprehensive local data is collated aimed at assessing the burden of gonorrhoea and evaluating the antibiotic resistance trend of *N. gonorrhoeae* in Bhutan.

2. MATERIALS AND METHODS

The laboratory data of urethral swab (US), cervical swab (CS) and high vaginal swabs (HVS) processed in the four hospitals with microbiology facilities during four years (2012-2015) were reviewed. All samples were processed using routine standard procedures by

trained medical laboratory staff. In these hospitals, female patients were sampled (CS/HVS/US) by the treating physicians and male patients were sampled (US) by laboratory staff. Microscopy of the sample was performed by Gram stain method and samples were cultured on Chocolate agar with sheep blood or modified Thayer-Martin agar when available. Isolates were identified by routine tests and use of sugars. Antibiotic susceptibility was tested according to the Calibrated Dichotomous Sensitivity Test (CDS) method and antibiotics were categorized as decreased susceptibility (DS), resistant (R) or susceptible (S) as per the CDS zone size interpretation criteria. Of the four laboratories, three take part in the external quality assessment with the Neisseria reference laboratory, WHO collaborating center for STI, Sydney and also report to the WHO Gonococcal Antimicrobial Surveillance Programme (WHO/GASP/SEAR Regional Reference Laboratory, New Delhi, India).

3. RESULTS

3.1 Burden and Demographic Trends of Gonorrhoea

Over the four years (2012-15), a total of 1,858 samples were processed in the four hospital; JDWNRH (60.2%, 1,119), PGH (17.7%, 329), CRRH (11.5%, 214) and ERRH (10.6%, 196). PGH recorded patients only from the year 2013. As shown in Fig. 1, there was an increasing trend in the number of cases tested in each hospital except for fewer cases tested in the JDWNRH in 2013. Most of the samples were from males and females accounted only for 1.2% (22) of the total samples. Of the 22 females, 11 were from JDWNRH, only in 2015 (no female patients in previous 3 years), four (1 in 2012 and 3 in 2014) from ERRH, seven (5 from 2012 and 2 from 2013) from CRRH and PGH has not tested any female samples in three years. The commonest age group was 20-30 years, 62.4% (1,160) and only 2.6% (48) were older than 50 years old (Table 1).

3.2 Clinical Samples and Laboratory Findings

All male samples were US. Of the 22 female samples, 18 were US, 3 were HVS and one was CS. In Gram stain, Gram-negative diplococci (GNDC) were seen in 80.7% (1,499) of samples and *N. gonorrhoea* was isolated in 64.5% (1,173)

of total cases or 78.25% of microscopy positives. No growths were seen in 17.3% (322) of the samples and in 2.0% (37) of cases no discharge were noted during sampling despite patients' complaints of urethral symptoms. The three hospitals - JDWNRH, PGH and CRRH reported high culture positive rates of 68.9% (750), 63.8% (210), and 67.3% (144) respectively but in ERRH culture positivity was only 36.7% (69) (Table 1). There was only 13.6% (3/22) culture positive rate in female samples. No samples with negative microscopy finding had growth of *N. gonorrhoeae*, but two such samples grew other organisms.

3.3 Trends in Antibiotic Resistance

Of the eight antibiotics that were routinely tested against *N. gonorrhoeae* in Bhutan, resistances against four antibiotics were very high; nalidixic acid 96.3% (1,129), ciprofloxacin 89.3% (1,046), penicillin G 87.8% (1,023) and tetracycline 72.9 (852). Fortunately, resistance against the other four antibiotics, including ceftriaxone (the current recommended antibiotic), was acceptably low; azithromycin 2.0% (23), cefpodoxime 1.9% (22), spectinomycin 0.8% (9) and ceftriaxone 0.2% (2) (Table 2).

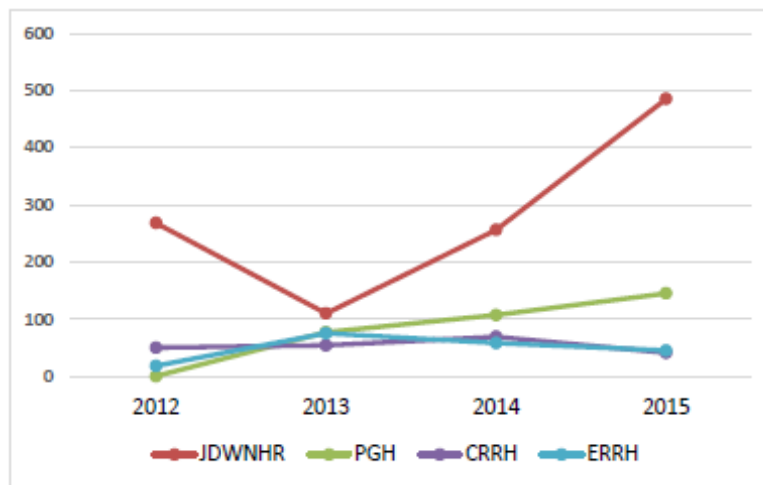


Fig. 1. Trends in number of gonorrhea cases in the four hospitals, 2012-15

JDWNRH: Jigme Dorji Wangchuck National Referral Hospital; PGH: Phuentsholing General Hospital; ERRH: Eastern Regional Referral Hospital; CRRH: Central Regional Referral Hospital

Table 1. Association between age and gonococcal infections and summary of laboratory findings (2012-15)

Characteristics		Overall		JDWNR		PGH		CRRH		ERRH	
		N	%	N	%	N	%	N	%	N	%
Age group (yrs.)	<19	86	4.6	52	4.7	12	3.7	10	4.7	12	6.1
	20-30	1160	62.4	699	62.5	235	71.4	117	54.7	109	55.6
	31-40	435	23.4	267	23.9	59	17.9	61	28.5	48	24.5
	41-50	129	6.9	76	6.8	17	5.2	20	9.4	16	8.2
	>50	48	2.6	25	2.2	6	1.8	6	2.8	11	5.6
Microscopy	GNDC seen	1499	80.7	879	78.6	282	85.7	182	85.1	156	79.6
	No GNDC	322	17.3	212	19.0	47	14.3	32	15	31	15.8
	No discharge	37	2.0	28	2.5	0	0.0	0	0.0	9	4.6
Culture	<i>N. gonorrhoeae</i> isolated	1173	64.5	750	68.9	210	63.8	144	67.3	69	36.7
	Not <i>N. gonorrhoeae</i>	2	0.1	2	0.2	0	0.0	0	0.0	0	0.0
	<i>gonorrhoeae</i>										
	No growth	645	35.4	337	31.0	119	36.2	70	32.7	119	63.3

JDWNRH: Jigme Dorji Wangchuck National Referral Hospital; PGH: Phuentsholing General Hospital; ERRH: Eastern Regional Referral Hospital; CRRH: Central Regional Referral Hospital; GNDC: Gram-negative diplococci

Table 2. Overall antibiotic susceptibility of *N. gonorrhoea* to different antibiotics in Bhutan, 2012-15

Antibiotics	Not tested		Decreased susceptibility		Resistant		Sensitive	
	N	%	N	%	N	%	N	%
Nalidixic Acid	40	3.4	1	0.1	1129	96.3	2	0.2
Ciprofloxacin	2	0.2	1	0.1	1046	89.3	123	10.5
Spectinomycin	1	0.1	0	0.0	9	0.8	1159	99.1
Ceftriaxone	1	0.1	15	1.3	2	0.2	1154	98.5
Penicillin	1	0.1	102	8.8	1023	87.8	39	3.4
Azithromycin	71	6.1	1	0.1	23	2.0	1067	91.8
Tetracycline	1	0.1	29	2.5	852	72.9	286	24.5
Cefpodoxime	41	3.5	9	0.8	22	1.9	1099	93.9

Cases of decreased susceptibility against some of these antibiotics were also noted. In all four hospitals, the resistance trend over the four years for nalidixic acid and ciprofloxacin remained consistently high (Table 3) but there were fluctuating levels of resistance to penicillin and tetracycline albeit with an overall rising trend.

The JDWNRH reported resistance up to 58.4% (104) for penicillin in 2012 and this increased to almost 100% (312) by 2015. Similarly, resistance to tetracycline was 41.9% (77) in 2012 but it increased to 83.2% (262) in 2015 (Fig. 2).

The resistance trend in three other hospitals remained high excepting in CRRH for tetracycline which saw decreasing resistance trend from 96.9% (31) in 2012 to 79.6% (39) and 83.3% (20) in 2014 and 2015 respectively (Table 3). The low level (<5%) of resistance against

ceftriaxone, azithromycin, spectinomycin and cefpodoxime has remained consistent over the four years (Fig. 3).

4. DISCUSSION

The laboratory data from the four large hospitals indicated that gonorrhea mostly affected the young sexually active men as expected. There was a steady increase of gonorrhea cases in the four laboratories during the study period (2012-15). This is consistent with the overall national annual increasing STI cases reported to the Ministry of Health over the recent years [10]. The highest number of cases from the JDWNRH is expected as it is the national referral hospital and caters to the capital city with the highest population [14]. PGH reported the second highest cases of gonorrhea and there are two

Table 3. Resistance trends of *N. gonorrhoea* against four antibiotics with high resistance by hospital (2012-15)

Antibiotics	Hospital	2012		2013		2014		2015	
		N	%	N	%	N	%	N	%
Nalidixic acid	JDWNRH	183	100.0	75	100.0	175	100.0	315	99.9
	PGH	NA	NA	52	100.0	60	98.4	100	100.0
	CRRH	0	0.0	31	81.6	51	100.0	24	100.0
	ERRH	8	100.0	17	94.4	22	100.0	16	94.1
Ciprofloxacin	JDWNRH	148	80.0	68	90.7	160	92.4	265	84.1
	PGH	NA	NA	52	100.0	60	98.4	100	100.0
	CRRH	31	86.9	36	94.7	42	84.0	21	87.5
	ERRH	8	100.0	17	94.4	22	100.0	16	94.1
Penicillin	JDWNRH	104	58.4	56	74.7	145	82.9	312	99.1
	PGH	NA	NA	51	98.1	59	96.7	91	91.0
	CRRH	32	100.0	38	100.0	48	96.0	48	100.0
	ERRH	8	100.0	18	100.0	22	100.0	15	88.2
Tetracycline	JDWNRH	77	41.9	49	66.2	98	56.0	262	83.2
	PGH	NA	NA	48	92.3	59	96.7	76	76.0
	CRRH	31	96.9	36	94.7	39	79.6	20	83.3
	ERRH	8	100.0	17	81.0	17	81.0	15	88.2

JDWNRH: Jigme Dorji Wangchuck National Referral Hospital; PGH: Phuentsholing General Hospital; ERRH: Eastern Regional Referral Hospital; CRRH: Central Regional Referral Hospital, NA: not available

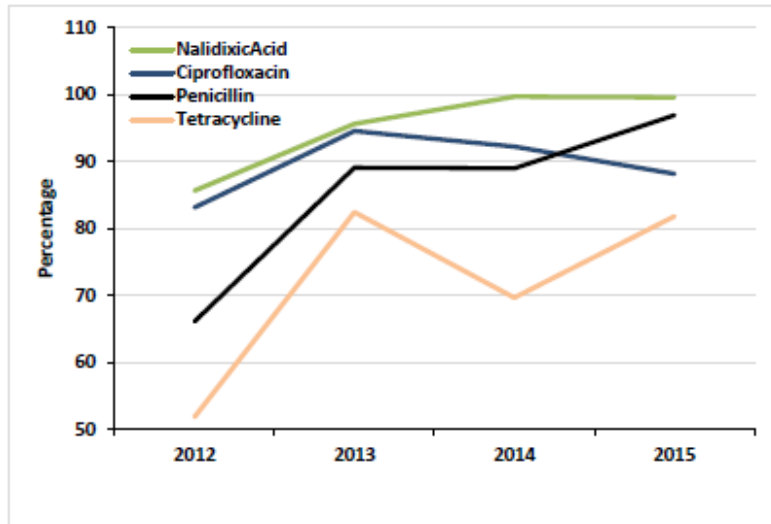


Fig. 2. *N. gonorrhoea* high resistance (>50%) trends against four antibiotics in Bhutan, 2012-15

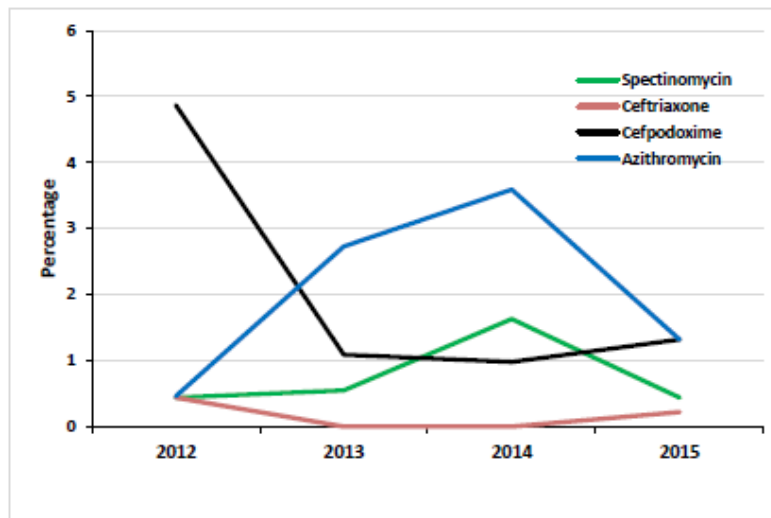


Fig. 3. *N. gonorrhoea* low resistance (<5%) trends against four antibiotics in Bhutan, 2012-15

plausible reasons for this; Firstly, Phuentsholing is the commercial hub of Bhutan with significant population movement, and secondly, there is a thriving sex trade, believed to be operating across the neighboring bordering town of Jaigaon, West Bengal state of India [15].

The four laboratories have received only 22 (1.2%) female samples compared to 98.8% 1,836 (98.8%) for males. This is far below the globally reported male to female gonorrhea case ratio of 1.14 in 2008 [1]. This could partially be due to more asymptomatic presentations in females ($\geq 50\%$) than in males ($\leq 10\%$) [2] thereby

not seeking treatment. Females also do not seek medical consultation given many of the symptoms of STI are seen as a sign of promiscuity [16], as a result, they may choose not to confide in anyone, even if they have an unusual discharge. In addition, females not being sampled by physicians due to the absence of discharge on examination may also have resulted in lesser female patients, since our study included only those that who have submitted samples to the laboratory for analysis. Fewer female cases frankly reveal that the concept of ‘seek and treat the partners’ as advocated in STI treatment guidelines [12] was

not practiced effectively. So there is a definite gap in the implementation of national treatment guidelines which needs to be reinforced through training and executive notifications to healthcare workers. This also warrants putting in place more women friendly clinics manned by female healthcare workers and increased awareness of signs and symptoms, risk factors and complications, preventive and control measures for STIs in general. A critical review of gaps in the current clinical practice and programmatic level would throw some light into instituting patient (particularly females) friendly strategies aimed at improving facilities and overcoming obstacles.

Although the recommended sample for females is the endocervical swab, the commonest sample received in the laboratories for female patients was US followed by HVS and CS. The culture positivity rate in females was very low compared to the males. Suboptimal samples might have resulted in the low culture positivity rate in females. Lower yields in females also could be due to prolonged time period between sample collection (in physician chambers) and laboratory processing compared to male samples that are collected in the laboratory and immediately inoculated onto culture media. About 2% of the cases, all males, with clinical urethritis had no discharge while attempting to be sampled. This could probably be due to non-gonococcal urethritis, which is typically caused by *Chlamydia trachomatis*, independently or concurrently with *N. gonorrhoea*. This calls for a detailed study on causative agents of STIs in Bhutan. Since the common aetiologies of STIs in Bhutan remain undetermined, syndromic management approach alone is not adequate as infections like gonorrhoea and chlamydia may not show any symptoms and patients may harbor multiple pathogens gradually leading into complications.

Findings of very high resistance to the previously used antibiotics are consistent with the international resistance trend in *N. gonorrhoea* [6,17]. Ceftriaxone, the current recommended therapy, has maintained high sensitivity since 2007 [13] until now in Bhutan. Resistance in Bhutan is lower than Myanmar and India but slightly higher than Nepal and Sri Lanka [18], probably due to a small number of isolates tested from Nepal and Sri Lanka. Because of increasing resistance against ceftriaxone worldwide, the USA, UK and Europe had stopped monotherapy with ceftriaxone and initiated dual therapy; combining ceftriaxone and azithromycin since

2011-2012 [19-21]. Considering such threat, *N. gonorrhoea*, still remaining susceptible to ceftriaxone in Bhutan is remarkable and beneficial to the resource-limited country.

5. CONCLUSION

In conclusion, the results from this study suggest an increasing burden of gonorrhoea in Bhutan. The resistance to antibiotics such as nalidixic acid, ciprofloxacin, penicillin G and tetracycline are very high. Fortunately, resistance against ceftriaxone (the current recommended antibiotic), azithromycin, spectinomycin and cefpodoxime were below the critical resistance rate of 5% for an antibiotic to be used as recommended treatment. Therefore, Bhutan can continue to use ceftriaxone as the therapy of choice but ongoing, well-coordinated, multi-site, antibiotic resistance surveillance, especially against ceftriaxone is required as resistance towards ceftriaxone continue to emerge and spread worldwide. Such ongoing surveillance would alert and guide in recommending continued use of ceftriaxone at the same or higher/multiple dosages or combined therapy or an overall change of recommended therapy to *N. gonorrhoea* in the future, as already recommended in some countries.

6. LIMITATIONS AND STRENGTHS

This study has a pertinent limitation of not having any data on patient presentations and clinical findings including complications since it involved the review of laboratory data only. Treatment details and outcomes could have added a lot of weight to the study. The biggest strength of the study is the inclusion of data from the four larger hospitals in the country, the only hospitals with microbiology culture facilities and equally representing different regions of the country. Therefore, the findings of this study practically represent data of the country.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Since only secondary data from routine laboratory records of the four hospitals without any personal identifying information were used, ethical clearance was not sought. However, administrative permission to use the data was sought from the four hospitals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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