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Misdiagnosis of Sexually Transmitted Diseases in Hong Kong Outpatient Private Healthcare

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Abstract

Background and objective: Early and accurate diagnosis is one of the critical requirements for successful management of all diseases. Yet, delayed diagnosis and misdiagnosis remain as vital problems, consequently impose adverse effects on patient treatment. Sexually transmitted disease (STD) is one of the most common infectious diseases, and more than one million of STD cases are acquired every day globally. Misdiagnosis of STD inevitably exists, therefore should not be overlooked. Being a medical diagnostic laboratory providing various STDs diagnosing service in Hong Kong, we aimed to determine the misdiagnosis rate of STDs and investigate the possible underlying cause. **Methods**: Specimens were collected for STD diagnosis from multiple clinics during 1 June 2021 to 20 October 2021 from different clinics and hospitals were included in the study. DNA extraction was performed using magnetic bead based method; then the extracted DNA was tested using the DiagCor GenoFlow™ STD Array kit to detect the existence of any targeted pathogens. **Results:** 1459 specimens were collected and included during the designated time period, with 643 specimens found to be positive with at least one targeted STD pathogen. 494 of these were found to be aligned with test ordered by physicians, and the remaining 149 positive cases had at least one pathogen detected but not requested to be tested by the physicians resulting in misdiagnosis. The overall misdiagnosis rate was determined to be 23.2% (149/643), with high frequency of misdiagnosis occurred to tests ordered for one to three pathogens detection. Also, *Ureaplasma urealyticum* and/or *Ureaplasma parvum* (UU/UP) was the commonest pathogen detected in this study. **Conclusion**: The findings suggested incorrect test selection made by physicians was one of the major reasons of STDs misdiagnosis in outpatient settings. To reduce diagnostic errors in STD diagnosis, physicians are encouraged to select and request test that allow detection of multiple pathogens, as co-infection of multiple pathogens in STD patients is commonly observed. The correct selec-

tion of test would not only benefit the patient, but also the public health.

Keywords

Sexually Transmitted Diseases (STDs), Diagnostic Error, Misdiagnosis, Incorrect Decision

1. Introduction

Numerous efforts have been devoted into developing novel diagnostic tests allowing faster and more precise diagnosis, which is critical in modern medicine. At present, about 70% of clinical decisions are based on the results of laboratory tests [1]. Indeed, the laboratory tests requests have escalated since 1920 [2] due to the aging population and clinical laboratory automation [3]. The increase of test requests reflects the importance of clinical laboratory service upon the improvement of patient outcome. Yet, diagnostic error still occurs from a day-to-day basis worldwide, imposing adverse impacts to both patients and healthcare systems. Studies had suggested that diagnostic errors happen about 12 million times per year in U.S. outpatients [4], and another study concluded that most people would experience a diagnostic error in their lifetime [5]. Incorrectness of ordering laboratory tests is one of the main factors leading to diagnostic errors [6]. Among all diagnostic errors made, mistakes in diagnosing sexually transmitted diseases (STDs) are common yet mostly overlooked.

Being one of the most common infectious diseases worldwide, STDs receive comparatively less attention as they are rarely fatal. However, they still impose great challenge to both patients and healthcare system if not diagnosed and treated swiftly [7]. Currently more than 30 different bacteria, viruses and parasites are known STDs pathogens, and STDs cases are increased in a rate of about 1 million per day globally estimated by the World Health Organization [8]. STDs are often missed by patients in the early stage as most are asymptomatic [9], while presentation of same non-specific symptoms by different pathogens causes diagnostic errors to be made in the form of misdiagnosis, as physicians might request diagnostic tests looking for the wrong pathogens. Therefore, accurate and timely diagnosis of STDs remains challenging, and is highly dependent on physicians' decisions.

Currently laboratory diagnosis of STDs includes mainly the following approaches: direct microscopy, isolation or/and culturing of pathogens, serology detection, and molecular detection of pathogens. Among the above approaches, many of the tests lack sensitivity, specificity and speed, in which only molecular diagnostic approaches can provide satisfactory performance on all these aspects [10]. While for molecular diagnostic methods, the two main methods are hybridization techniques and amplification techniques (e.g. PCR, qPCR). Comparing the two molecular methods, hybridization techniques offer the advantages of detecting a larger target panels in a single setting, in which amplification tech-

niques mostly detect 3 to 4 targets in a single reaction even using multiplex settings.

As a medical diagnostic laboratory, we provide STDs molecular diagnostic test using the DiagCor GenoFlow™ STD Array kit, which is capable of detection of eight STDs pathogens (ST1 to ST8) simultaneously for each specimen. This kit allows a more comprehensive and detailed diagnostic result provided in a single reaction, comparing to other STDs diagnostic kits available e.g. BD MAX™ CT/GC and CT/GC/TV assays, Qiagen digene HC2 CT/GC DNA test, and SolGent DiaPlexQ™ STI 12 Detection Kit. The lowest detection limit of GenoFlow™ STD Array kit is 50 copies of each STD pathogen per reaction. No cross-reactivity was found as claimed by the manufacturer. In order to ensure the performance of our laboratory and the test kits, we have participated the Quality Control for Molecular Diagnostics (QCMD) and continuously achieving 100% accuracy in identifying the pathogens provided by the association each year. Clinics and physicians are provided and can select a series of eight STD tests from our test menu, namely the ST1 to ST8, which provide screening service of different number of pathogens with different test fees. ST1 is the cheapest test in the series and only provide a test report with result of one pathogen chosen by physician; ST2 with report of two chosen pathogens and a slightly increased fee; and up till ST8 which provide a report with result for all targeted pathogens by the test and the highest fee among the eight STD tests. Owing to this test arrangement, we are able to identify if any discrepancy exist between pathogen(s) chosen to be tested by physicians and the pathogen(s) detected by the test kit. A long-time concern is that physicians could have requested tests targeting the wrong pathogen(s), and the detected pathogen(s) was not reported to the physicians. As a result, physicians would make inappropriate diagnosis and provide improper treatments to patients with STD. In the present study, we collected the STDs diagnostic test results obtained from 1 June 2021 to 20 October 2021 from more than 20 clinics and hospitals, and use this data to determine and evaluate the diagnostic error rate of STDs due to the incorrect test ordered by physicians.

2. Materials and Methods

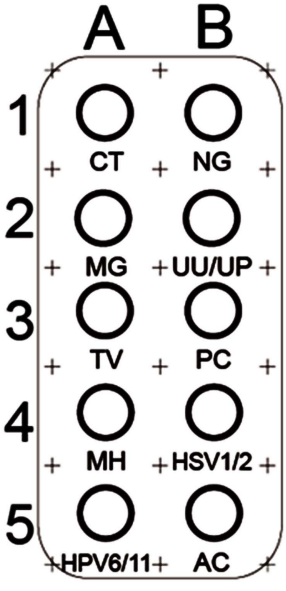
2.1. Specimen Type and DNA Extraction

Clinical specimens for STDs molecular diagnostic test included urine, liquid based cytology specimen (e.g. SurePath, ThinPrep), and swabs of different anatomical sites (cervix, glan, anal, throat, etc.). Then DNA extraction was performed either by autonomic system (DiagPuro™ Nucleic Acid Extraction System, (DiagCor Life Science, Hong Kong, China) or magLEAD® 6 gC/magLEAD® 12 gC system (Precision System Science, Japan), and manually using Qiagen QIAamp® Blood Mini kit if repeat extraction was needed. DNA extraction was run according to the Instruction for Use (IFU) provided by the kit manufacturers.

2.2. Molecular Detection of STD Pathogens

Molecular detection of STD pathogens was performed using the DiagCor GenoFlow™ STD Array CE-IVD Test Kit (DiagCor Life Science, Cat.No: 92010, Hong Kong SAR, China), based on the Polymerase Chain Reaction (PCR) and "flow-through" hybridization technology, which is capable of detecting the following pathogens in a single reaction: *Trichomonas vaginalis* (TV), *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG), *Mycoplasma hominis* (MH), *Neisseria gonorrhoeae* (NG), *Ureaplasma urealyticum* and/or *Ureaplasma parvum* (UU/UP), HPV type 6 and/or type 11, and HSV type 1 and/or type 2.

All tests were performed according to IFU provided by the kit manufacturer. As in typical nucleic acid amplification testing (NAT), compartmentalization/clearly designated areas and appropriate procedures and controls were applied to prevent cross-contamination. In brief, the extracted genomic DNA of target pathogen(s) was amplified by biotinylated primers using PCR with PCR conditions indicated in Table 1. The amplicons were subsequently hybridized to the pathogen-specific capturing probes coated on nylon membrane via "Flow-through" hybridization. Amplicons were detected actively and formed duplexes with the probes, followed by a stringent wash and signal development to detect formation of any duplexes. Signals developed would be presented as a dark spots on the nylon membrane, while the location of the dark spot suggested the detection of the particular STD pathogen (Figure 1). Multiple spots indicated the detection of multiple pathogens in the tested sample. The whole "Flow-through" hybridization and signal development procedure was performed using the DiagCor FT^{PRO} Flow-through System (DiagCor Life Science, Hong Kong SAR, China).



Target pathogens (Abbreviation)		
Protozoa	*Trichomonas vaginalis*	TV
Bacteria	*Chlamydia trachomatis*	CT
	Neisseria gonorrhoeae	NG
	Mycoplasma genitalium	MG
	Mycoplasma hominis	MH
	Ureaplasma urealyticum	UU
	Ureaplasma parvum	UP
Virus	Herpes simplex virus 1	HSV1
	Herpes simplex virus 2	HSV2
	Human papillomavirus type 6	HPV6
	Human papillomavirus type 11	HPV11

Scientific Controls (Abbreviation)		
Controls	Positive Control	PC
	Amplification Control	AC

Figure 1. Target pathogens detected in GenoFlow™ STD Array kit and signal position presented on the nylon membrane (grids shown for illustration purpose only), indicating the test kit was capable to detect up to 11 pathogens with 8 signals in a single test.

Table 1. Details of thermos profile for PCR DNA amplification.

Stage	Step	Temperature (˚C)	Time
Hold	Initial denaturation	95	10 min
43 cycles	Denature	95	30 sec
	Annealing/Extension	60	1.5 min
Hold	Final Extension	72	7 min
Hold	Final hold	4	∞

3. Results

During the study period, a total of 1459 STD molecular diagnostic tests were ordered by physicians according to our test menu (ST1 to ST8) and performed, with 565 samples from male and 894 samples from female. Among ST1 to ST8 test ordered by physicians, ST7 which allowed screening for seven selected pathogens in one sample had the highest frequency of being ordered (24.2%), and with ST3 which allowed screening of three selected pathogens being the least ordered (1.6%) (Figure 2). Out of these 1459 tests, 641 were performed with urine samples, 380 with liquid based cytology samples, and 438 with swabs of various anatomical sites. From these 1459 samples ordered, 816 samples were tested negative for all eight listed pathogens and the remaining 643 samples were tested positive for at least one pathogen. For the 643 samples, 471 samples were tested to be positive for infection of single pathogen; whereas 172 (26.7%) samples were tested to be positive of co-infection of multiple pathogens (Table 2). Female patients was found to have a higher positive rate comparing to male patients, with a rate of 51.3% (459/894) and 32.6% (184/565) correspondingly. In terms of pathogens, *Ureaplasma urealyticum* and/or *Ureaplasma parvum* was the most frequently detected pathogen among positive samples with a detection rate of 76.7% (493/643), followed by *Mycoplasma hominis* with a rate of 17.7% (114/643) (Figure 3).

Among all requested tests, *Chlamydia trachomatis* (CT) was the most common pathogen ordered to test (n = 1192) by physicians (Table 3), explained by the high prevalence of chlamydia in sexually active women of all age in Hong Kong [11]. The second most common pathogen required to test was *Ureaplasma urealyticum* and/or *Ureaplasma parvum* (UU/UP) (n = 1093), followed by Neisseria *gonorrhoeae* (NG) (n = 1090), while HPV 6/11 was the least ordered (n = 210) (Table 3).

Among these 643 pathogen positive samples, 494 samples were tested positive for pathogen(s) matching with the pathogens(s) requested by test ordered; whereas the remaining 149 samples were positive for pathogen(s) which were not ordered to be tested by physicians, hence "mismatched". Amid the eight test types (ST1 to ST8), ST2 presented highest mismatching rate with 77.4% (65/84) positive cases not reported to physicians (Figure 4). The commonest combination of pathogens ordered for ST2 test by physicians was *Chlamydia trachomatis*

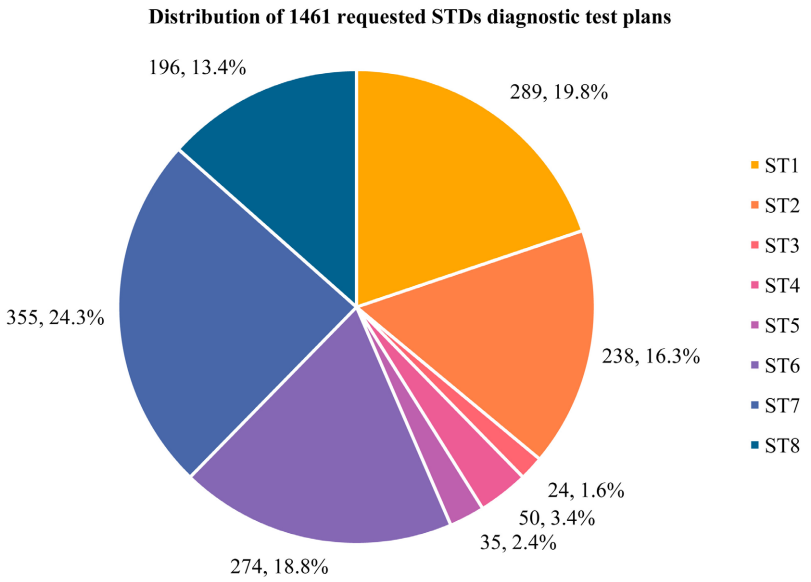


Figure 2. The distribution of ST1 to ST8 test plans ordered by physicians during the study period. This figure showed that more than 50% of physicians ordered ST6, ST7 or ST8 test plans, indicating that it was a common practice for most of physicians to examine for multiple STDs in patients. The figure also showed a tremendously low requesting rate of ST3, ST4, and ST5, with approximately 7% requesting rate from physicians.

Table 2. Results of STDs molecular diagnostic tests collected from June 2021 to September 2021. Information and result categorized based on test requested. 643 samples were tested positive for STD pathogen(s), with 471 samples positive for one pathogen and 172 samples positive for multiple pathogens.

Form of test*	No. of samples	No. of STD(s) positive samples	No. of samples detected with one pathogen	No. of samples detected with multiple pathogens
ST1	287 (19.7%)	115 (40.1%)	92	23
ST2	238 (16.3%)	84 (35.3%)	61	23
ST3	24 (1.6%)	12 (50%)	6	6
ST4	50 (3.4%)	23 (46%)	17	6
ST5	35 (2.4%)	19 (54.3%)	11	7
ST6	275 (18.8%)	108 (39.3%)	75	33
ST7	353 (24.2%)	174 (49.3%)	132	42
ST8	197 (13.5%)	108 (54.8%)	77	31
Total	**1459**	**643**	**471**	**172**

*ST1: one specific STD pathogen ordered to test and reported; ST2: Two specific STD pathogen ordered to test and reported; ST3: Three specific pathogens ordered to test and reported; ST4: Four specific pathogens ordered to test and reported; ST5: Five specific pathogens ordered to test and reported; ST6: Six specific pathogens ordered to test and reported; ST7: Seven specific pathogens ordered to test and reported; ST8: All eight listed pathogens ordered to test and reported.

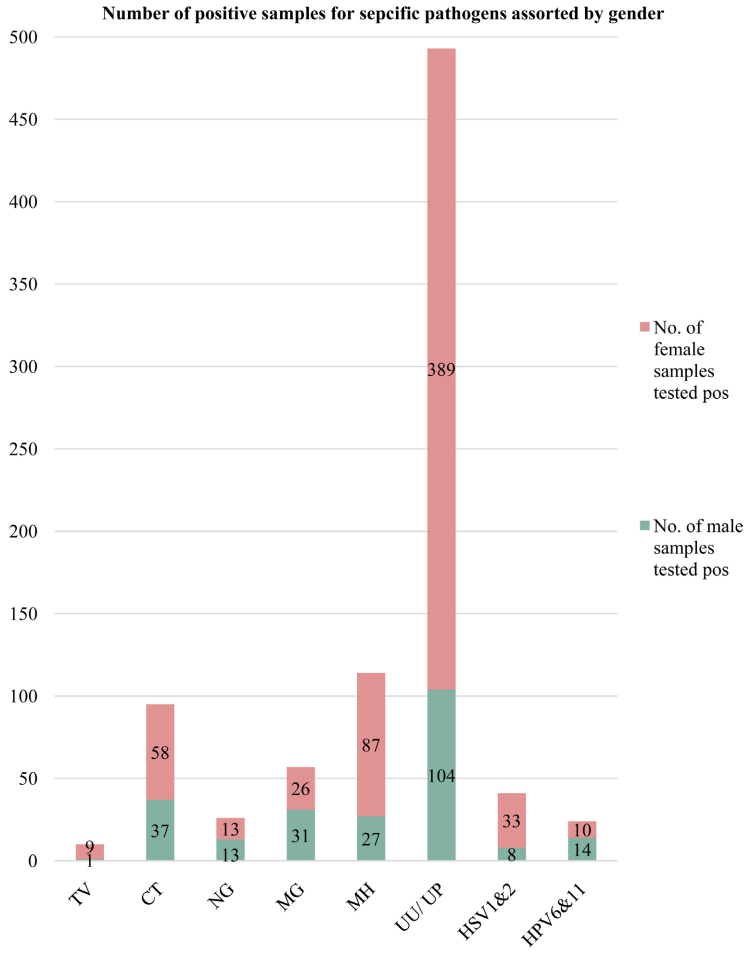


Figure 3. Detection of pathogens in the recruited specimens. Among the 1459 patients tested, the most prevalent pathogens, for both men and women, were UU/UP, followed by *M. hominis* and *C. trachomatis*.

Table 3. Test result categorised based on targeted pathogens, with number of samples with detected pathogen aligned with requested tests; number of samples with detected pathogen not aligned with requested tests.

Targeted pathogen*	No. of samples tested	No. of positive samples	Detected pathogen aligned with requested test	Detected pathogen not aligned with requested test	Misdiagnosis rate
TV	734	10	8	2	20%
CT	1192	95	86	9	9.5%
NG	1090	26	24	2	7.7%
MG	920	57	34	23	40.4%
MH	911	114	85	29	25.4%
UU/UP	1093	493	412	81	16.4%
HSV1 & 2	757	41	28	13	31.7%
HPV6 & 11	210	24	3	21	87.5%

* *Trichomonas vaginalis* (TV); *Chlamydia trachomatis* (CT); *Mycoplasma genitalium* (MG); *Mycoplasma hominis* (MH); *Neisseria gonorrhoeae* (NG); *Ureaplasma urealyticum* and/or *Ureaplasma parvum* (UU/UP); HSV type 1 and/or type 2 (HSV1 & 2); HPV type 6 and/or type 11 (HPV6 & 11).

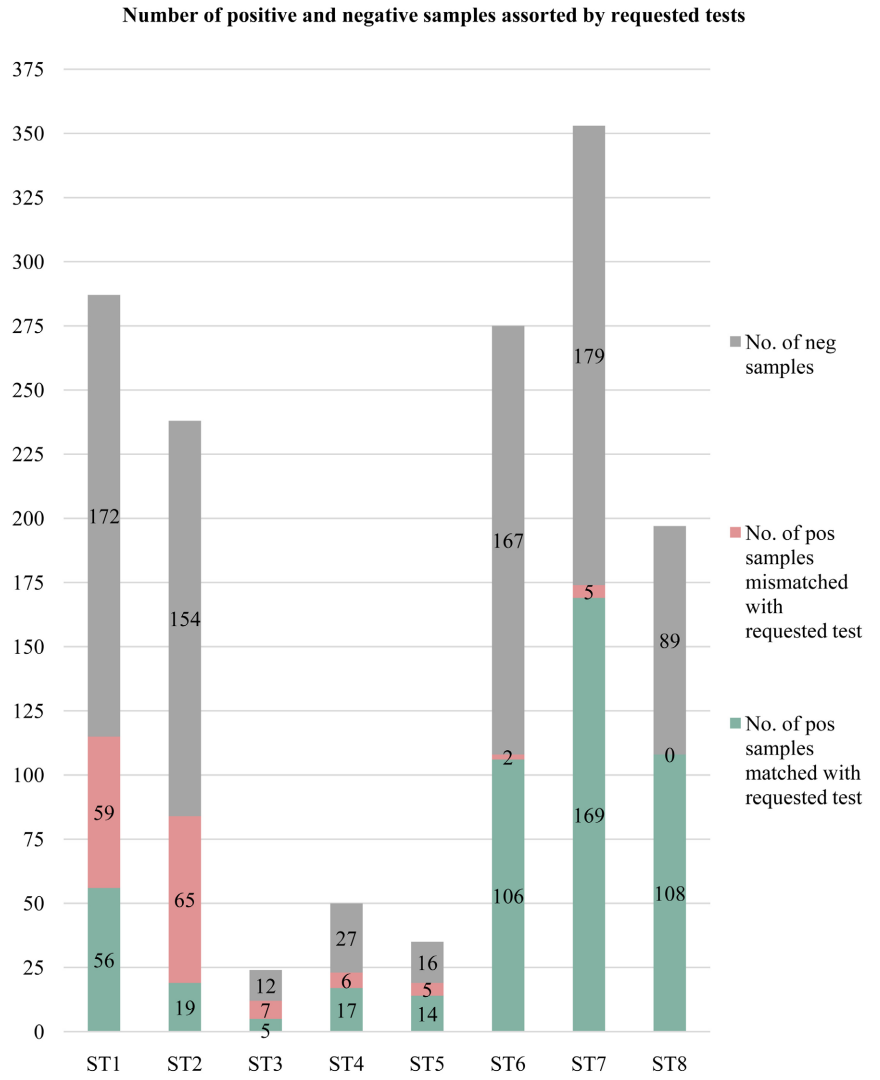


Figure 4. Stacked bar-chart of the 1459 tests categorised based on test plan requested (ST1 to ST8), with number of negative tests, positive samples with matched or unmatched tests requested. A significant difference in number of positive samples mismatched with requested tests between ST1 and ST2 and ST6 and ST7 could be observed.

and *Neisseria gonorrhoeae*, which made up 192 tests out of the 238 tests (80.7%) (data not shown). Among these 192 tests, only six tests were reported as positive to the physicians, with 56 tests observed to be mismatched. For these 56 mismatched tests, 33 (58.9%) of them were positive for *Ureaplasma urealyticum* and/or *Ureaplasma parvum* (Data not shown), suggesting physicians might have neglected the common infection of UU/UP in Hong Kong, or the infections of UU/UP could have been asymptomatic causing the physicians to have unnoticed.

On the contrary, the 197 ST8 test request, approximately 13.5% of all tests requested, presented no misdiagnosis, as ST8 allowed the screening of all targeted pathogens. While for ST6 and ST7, accounted for 43.0% of all STD tests included in this study, covering only 6 and 7 pathogens out of the 8 targeted pathogens,

only presented a combined misdiagnosis rate of 2.5% (7/282), imposing a much superior diagnostic capability and far lower misdiagnosis rate comparing to ST1 to ST5 (**Figure 4**).

4. Discussion

In our study, *Ureaplasma urealyticum* and/or *Ureaplasma parvum* was identified as the commonest STD pathogen detected, and this finding was concordant with a previous study conducted in Hong Kong [11]. Ureaplasma are self-replicating, free-living microorganisms and can only be identified via molecular approaches, such as flow-through hybridisation employed in this study or PCR, and the growth in usage of these techniques in diagnostic lab has resulted in increasing number of people being diagnosed as infected with these microorganisms. Even so, *Ureaplasma urealyticum* and/or *Ureaplasma parvum* was frequently omitted by physicians for ST1 and ST2 tests and identified as the most misdiagnosed pathogen for ST1 and ST2 tests by this study (data not shown). The omission of UU/UP by physicians for ST1 and ST2 tests could be due to Ureaplasma ability of surviving in the mucosa of urogenital tract of healthy individuals without displaying any symptoms.

U. urealyticum is a common cause of urethritis and prostatitis in male [12]; and for female Ureaplasma infection can lead to endometritis and bacterial vaginosis, or chorioamnionitis and premature rupture of membrane in pregnant individuals. Additionally, infection of Ureaplasma in man is also associated with fertility problem, possibly by affecting the number of active sperm [13], also a prolonged ureaplasma infection in female could result in infertility [14]. Transmission of Ureaplasma can be achieved by genital-to-genital or oral-to-genital sexual activities, and also from mother-to-infant through three ways (transplacental; vertical transmission; horizontal or nosocomial transmission). Considering the outcomes of Ureaplasma infection, it is important to make early diagnosis and treatment for the infected individuals.

Another major and more important observation in this study was the high rate of incorrect test selection made by physicians and the high amount of "mismatched" result, which could lead to delayed diagnosis or worse misdiagnosis as the detected pathogens would not be reported to the physicians. Among the tests, tests ST1 and ST2 presented the most misdiagnosis cases, 59 cases (20.6%, 59/287) and 65 cases (27.3%, 65/238) (**Figure 4**) respectively. While an unexpected low requesting rate for ST3, ST4, and ST5 was observed in this study, which unfortunately limited the observation and interpretation.

The high misdiagnosis rate observed could be explained by the high requesting rate of tests ST1 and ST2. These two tests only provide diagnosing service for one or two pathogens selected by the clinician; hence, a successful diagnosis fully depends on choosing the correct pathogens for the tests which require the clinician to be both experienced and knowledgeable about STDs in Hong Kong. At the same time, co-infection of multiple pathogens is common in STD patients;

the tests ST1 and ST2 would not be able to present the full clinical picture to the physicians. This study has clearly demonstrated wrong decisions made or wrong tests selected by physicians being a major reason for STDs misdiagnosis, which brings about the delayed treatment in patients.

Some may question if the ST1 and ST2 tests only provide such a limited diagnostic value, why such tests remain available for physicians. The truth is the original purpose of both tests were not for either diagnosing or screening of any STDs, but for physicians to confirm the treatment effect for STDs patients undergoing or finished treatment e.g. antibiotic treatment efficiency in treating Ureaplasma. For diagnosing and screening of STDs, it is a common practice that patients should be offered tests for multiple pathogens to avoid misdiagnosis, and in this study the low misdiagnosing rate observed in tests ST6 and ST7 proven the importance of that. This suggested misdiagnosis could also be the result of physicians misunderstanding the purpose and functions of diagnostic tests. Another possible explanations for the high rate of misdiagnosis, or high amount of incorrect tests ordered probably because patients often display asymptomatic or are concurrently co-infected by other pathogens that make overlook by physicians. As presented in **Table 1**, out of the 115 positive ST1 tests 23 were positive for multiple pathogens, suggesting 20% of ST1 positive cases would have additional STD infection being unnoticed. This once again indicated it is important to remember the high frequency of co-infection with various STD pathogens in a single patient, and ordering tests for multiple pathogens would be essential for the patients' and public health interest.

The major limitation for this study was unable to provide a full assessment of misdiagnosing rate for the whole panel of STDs tests provided especially for ST3 to ST5, which were rarely ordered, and only composed about 7% of all tests requested during the study period, and the data obtained were insufficient compared to the other tests. It is believed that the misdiagnosing rate should decrease across the whole panel of STDs tests, from ST1 to ST8, but the data for ST3, ST4 and ST5 obtained were insufficient to make a proper comparison with the other tests.

Despite the limitation mentioned, this study had provided a precious hindsight in STDs diagnosis in outpatient settings in Hong Kong, and we believe the actual misdiagnosing rate could be even higher in reality. As observed in this study many physicians in Hong Kong still order STDs diagnosing tests with limited number of pathogens for their patients, while the test kit currently used in our lab allowed us to detect a panel of eight common pathogens and identify the misdiagnoses, other diagnostic laboratories might not have the same capability in identifying the problem and many misdiagnosing cases would have gone unnoticed. It is vital that the physicians should always assume their patients could have multiple STDs at the same time and request a more comprehensive test which detects more pathogens. On the other hand, diagnostic laboratories should adopt a low cost STDs diagnostic test which allow detection of multiple pathogens simultaneously e.g. multiplex qPCR-based test in a sample, and encourage

physicians to order such a test for a panel of pathogen screening to diminish the chance of misdiagnosis. Once again accurate diagnosis is crucial not just only for the patients with STD, but also to public health and control of STDs transmission in the society.

5. Conclusion

In conclusion, this study successfully determined a high discrepancy between the ordered test for pathogens by physicians and the actual detection of pathogens in outpatient settings, which in turn affected the diagnosis of STD and proper treatment to patients. Furthermore, incorrect decisions made by physicians are the major causes of misdiagnosis observed. This study illustrated the importance of physicians being not overconfident nor being bias when making diagnostic decisions, and also the possibility of need to review and update of current STD diagnosis practices and educate physicians on prevalence of STDs and correct usage of different diagnostic tests. Once again, rapid and accurate diagnosing is essential in managing all kinds of diseases, and diagnostic errors should be avoided at all cost.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding publication of this article.

Patient Consent for Publication

Not required.

References

[1] Forsman, R.W. (1996) Why Is the Laboratory an Afterthought for Managed Care Organizations? *Clinical Chemistry*, **42**, 813-816. https://doi.org/10.1093/clinchem/42.5.813

[2] Wians, F.H. (2009) Clinical Laboratory Tests: Which, Why, and What Do the Results Mean? *Laboratory Medicine*, **40**, 105-113. https://doi.org/10.1309/LM404L0HHUTWWUDD

[3] Armbruster, D.A., Overcash, D.R. and Reyes, J. (2014) Clinical Chemistry Laboratory Automation in the 21st Century-Amat Victoria Curam (Victory loves Careful Preparation). *The Clinical Biochemist Reviews*, **35**, 143.

[4] Singh, H., Meyer, A.N. and Thomas, E.J. (2014) The Frequency of Diagnostic Errors in Outpatient Care: Estimations from Three Large Observational Studies Involving US Adult Populations. *BMJ Quality & Safety*, **23**, 727-731. https://doi.org/10.1136/bmjqs-2013-002627

[5] Balogh, E.P., Miller, B.T. and Ball, J.R. (2015) Improving Diagnosis in Health Care.

National Academies Press (US), Washington DC. https://doi.org/10.17226/21794

[6] Schiff, G.D., Hasan, O., Kim, S., Abrams, R., Cosby, K. and McNutt, R.A. (2009) Diagnostic Error in Medicine: Analysis of 583 Physician-Reported Errors. *Archives of Internal Medicine*, **169**, 1881-1887. https://doi.org/10.1001/archinternmed.2009.333

[7] Van Gerwen, O.T., Camino, A.F., Sharma, J., Kissinger, P.J. and Muzny, C.A. (2021) Epidemiology, Natural History, Diagnosis, and Treatment of *Trichomonas vaginalis* in Men. *Clinical Infectious Diseases*, **73**, 1119-1124. https://doi.org/10.1093/cid/ciab514

[8] Fuchs, W. and Brockmeyer, N.H. (2014) Sexually Transmitted Infections. *JDDG: Journal der Deutschen Dermatologischen Gesellschaft*, **12**, 451-464. https://doi.org/10.1111/ddg.12310

[9] Khan, A., Plummer, D., Hussain, R. and Minichiello, V. (2007) Sexual Risk Assessment in General Practice: Evidence from a New South Wales Survey. *Sexual Health*, **4**, 1-8. https://doi.org/10.1071/SH06012

[10] Muralidhar, S. (2015) Molecular Methods in the Laboratory Diagnosis of Sexually Transmitted Infections. *Indian Journal of Sexually Transmitted Diseases and AIDS*, **36**, 9-17. https://doi.org/10.4103/0253-7184.156686

[11] Chan, C.L., Chan, W.Y., Cheng, C.H.K. and Xia, P. (2016) What Is Missing in STD Screening in Hong Kong? *Current Research in Medicine*, **7**, 13-18. https://doi.org/10.3844/amjsp.2016.13.18

[12] Zhang, N., Wang, R., Li, X., Liu, X., Tang, Z. and Liu, Y. (2014) Are Ureaplasma spp. a Cause of Nongonococcal Urethritis? A Systematic Review and Meta-Analysis. *PLOS ONE*, **9**, e113771. https://doi.org/10.1371/journal.pone.0113771

[13] Huang, C., Zhu, H.L., Xu, K.R., Wang, S.Y., Fan, L.Q. and Zhu, W.B. (2015) Mycoplasma and Ureaplasma Infection and Male Infertility: A Systematic Review and Meta-Analysis. *Andrology*, **3**, 809-816. https://doi.org/10.1111/andr.12078

[14] Piscopo, R.C., Guimarães, R.V., Ueno, J., Ikeda, F., Jarmy-Di Bella, Z.I., Girão, M.J. and Samama, M. (2020) Increased Prevalence of Endocervical Mycoplasma and Ureaplasma Colonization in Infertile Women with Tubal Factor. *JBRA Assisted Reproduction*, **24**, 152-157. https://doi.org/10.5935/1518-0557.20190078