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Metformin and Antimicrobial Agents Impacts on Bacterial Isolates from Vaginal Infections of women with or without Polycystic Ovarian Syndrome

A project submitted to the council of the college of pharmacy in partial fulfilment of requirement for the degree of bachelors of pharmacy B.P.S

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

﴿وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ﴾

سورہ یونس الایہ (10)

"صدقَ اللهُ العليُّ العظيم"

الاهداء

قال تعالى : (وَأَن لَّيْسَ لِلْإِنسَانِ إِلَّا مَا سَعَى وَأَنَّ سَعْيَهُ سَوْفَ يُرَى)

إلهي لا يطيب الليل إلا بشكرك ولا يطيب النهار إلا بطاعتك ..

ولا تطيب اللحظات إلا بذكرك .. ولا تطيب الآخرة إلا بعفوك ... ولا تطيب الجنة إلا برؤيتك الله جل جلاله

إلى من بلغ الرسالة وأدى الأمانة .. ونصح الأمة .. إلى نبي الرحمة ونور العالمين سيدنا محمد (صلى الله عليه واله وسلم)

إلى رجل الكفاح ، إلى من زرع القيم والمبادئ الإسلامية ، إلى من أفنى زهرة شبابه في تربية أبنائه ... والدي الحبيب

إلى ملاكي في الحياة .. إلى معنى الحب وإلى معنى الحنان والتفاني .. إلى بسمة الحياة وسر الوجود إلى من كان دعائها سر نجاحي وحنانها بلسم جراحي إلى أغلى الحبايب.... أمي الحبيبة

إلى منارة العلم والعلماء إلى الصرح الشامخ ....

جامعة بابل كلية الصيدلة

إلى الذين حملوا أقدس رسالة في الحياة إلى الذين مهدوا لنا طريق العلم والمعرفة .. أساتذتنا الأفاضل

List of contents:

List of Abbreviations:

Abbreviations	Meaning
PCOS	Polycystic ovary syndrome
DPP-4	4dipeptidyl peptidase 4
AMPK	Adenosine monophosphate-activated protein kinase
mG3PDH	Mitochondrial glycerol 3-phosphate dehydrogenase
CC	Clomephine citrate
TRPA1	Transient receptor potential ankyrin 1
IGF-1	Insulin-like growth factor 1
TNF-α	Tumor necrosis factor-α

Abstract:

The continuous increase in the incidence of bacterial resistance to existing antibiotics represents a worldwide health burden. A surrogate strategy to combat such crisis is to find compounds that restore the antimicrobial activity of the already existing antibiotics against multidrug resistant bacteria. Metformin is a commonly used antidiabetic medication. It has proven benefits in other diseases including cancer, aging-related and infectious diseases. In this study, the potential effect of metformin as an adjuvant therapy to antibiotics was investigated. **Methods:** In this research, 87 samples collected from patients (female) suffering from vaginal infections attending the Gynecology and obstetrics teaching hospital in Hillah, then diagnosed bacteria including: *E.coli, Klebsiella pneumoniae* and *Pseudomonas aeroginosa*, the diagnosis, identification and antibiotic sensitivity tests were performed according to standard guidelines after that, effect of metformin mixed with other tested antibiotics was tested against the isolated bacterial, antibacterial activity was measured. **Results:** metforming as assistant therapy indicated variable effect against bacterial isolates, effect on *Klebsiella pneumoniae* and *Pseudomonas aeroginosa* was clearly increased compared to effect of antibiotics only, while regarding *E.coli*, the effect of metformin mixed with tested antibiotics was lower obviously than on other isolated bacteria. **Conclusion:** Metformin exerts an adjuvant antibacterial effect; thus, it could be a possible candidate as supporting therapy to reduce antimicrobial resistance.

Introduction:

Metformin, an insulin-sensitizing biguanide used to treat type 2 diabetes, has been shown to be as effective as insulin or sulfonylureas when used as monotherapy[1,2] . In conjunction with diet, metformin reduces fasting glucose concentration by 2.78 to 3.90 mmol/L (50 to 70 Mg/dL), which corresponds to a 1.3% to 2.0% reduction In hemoglobin A1c values [1, 3, 4, 5,6] The efficacy of metformin monotherapy has been shown to be independent of age, body weight, duration of diabetes, and insulin and C-peptide levels[1،3]

Metformin therapy should be initiated with a single dose of medication (usually 500 mg) taken with the patient's largest meal to prevent gastrointestinal symptoms. Gastrointestinal sympom generally disappear within 2 weeks of treatment[7,8] . Medication doses may be increased by 500-mg increments every 1 to 2 weeks, as indicated by glycemic control, until a desirable blood glucose level or the maximal recommended daily metformin dose of 2550 mg is reached [3, 9].

The dose for glucose-lowering efficacy is usually in the range of 500–2000 mg/day. There is no standard dosage rigmen for the management of hyperglycemia in patients with type 2 diabetes.The dosage of metformin must be individualized for every patient considering effectiveness and tolerance while not exceeding the maximum recommended daily doses (2550 mg in adults and 2000 mg in pediatric patients >10 years of age), Patients that are receiving immediate-release metformin treatment may be switched to extended form once daily with the same total daily dose (up to 2000 mg daily). In the case of renal impairment, the dosage of metformin must be adjusted

Mechanism and induction of metformin:

- The glucose-lowering effects of metformin are mainly a consequence of reduced hepatic glucose output (primarily through inhibition of gluconeogenesis and, to a lesser extent, glycogenoglucos and increased insulin-stimulated glucose uptake in skeletal muscle and adipocytes [9, 10, 11,12]

The exact mechanism through which metformin reduces hepatic glucose production remains unclear, but its primary site of action appears to be hepatocyte mitochondria, where it disrupts respiratory chain oxidation of complex I substrates (for example, glutamate) [13,14]. Inhibition of cellular respiration decreases gluconeogenesis [14] and may induce expression of glucose transporters and, therefore, glucose utilization [15]. It is not clear whether metformin acts on mitochondrial respiration directly by slow permeation across the inner mitochondrial membrane [13]. or by unidentified cell-signaling pathways, It has been suggested that biguanid membran specifically and competitively to divalent cation sites on proteins, thus interfering with intracellular handling of calcium especially in the mitochondria [16,17]

PCOS patients suffer from insulin resistance and hyperinsulinemia [18]. Metformin has been used for pcos treatment [19] for treating the metabolic abnormalities of pocs. A recent meta-analysis [14] demonstrated that metformin could decrease testosterone and insulin level in women with pcos

The cardiovascular protective effects of metformin could be explained by the reduced level of LDL cholesterol [20], the limitation of weight gain, [21] and the improvement of oxidative stress, inflammatory response, and the endothelial cell function [22].

Metformin has a better protective effect on the domain of vebral learning, working memory, and executive function than other diabetic treatments [23].

Metformin may have special benefits in overweight patients with type 2 diabetes Metformin does not affect body mass index or decreases body weight in obese patients with [4, 7] and without [8, 24] diabetes. Significant reductions in total body fat and visceral fat have been observed in women with preexistent abdominal or visceral obesity who are treated with metformin [8]. Weight loss during metformin treatment has been attributed to decreased net caloric intake [13], probably through appetite suppression, an effect that is largely independent of gastrointestinal side effects of metformin (such as nausea and diarrhea) [17]. Reduction in hyperinsulinemia related to reduced insulin resistance may have an additive effect on weight reduction in obese insulin-resistant persons [9, 25].

Side Effects of Metformin:

Side effects of metformin are mostly limited to digestive tract symptoms, such as diarrhea, flatulence, and abdominal discomfort [1,5, 6,7] . These symptoms are dose dependent and can usually be avoided by slow titration and, in some cases, reduction of the dose[26] About an of patients cannot tolerate treatment because of gastrointestinal side effects [5, 26, 7].The mechanisms of these gastrointestinal side effects remain unclear but probably are related to accumulation of high amounts of metformin in the intestinal tissue [28],with subsequent elevation of local lactate production.these common side effects of metformin happen in more than 1 in 100 people. There are things you can do to help cope with them: Feeling sick (nausea), Being sick (vomiting), Diarrhoea, Stomach ache, Loss of appetite, metallic taste in the mouth

Vitamin B12 deficiency:

Taking metformin can cause vitamin B12 deficiency. Such as Feel very tired, muscle weakness, sore, red tongue, mouth ulcers, problems with your vision, pale or yellow skin (this may be less obvious on brown or black skin, your doctor can check your vitamin B12 serum levels. If they are too low, they may prescribe B12 vitamin supplements

Contraindications:

Ketoacidosis

Cardiac failure

Chronic kidney disease (CKD)

Hepatic failure and cirrhosis

Respiratory insufficiency

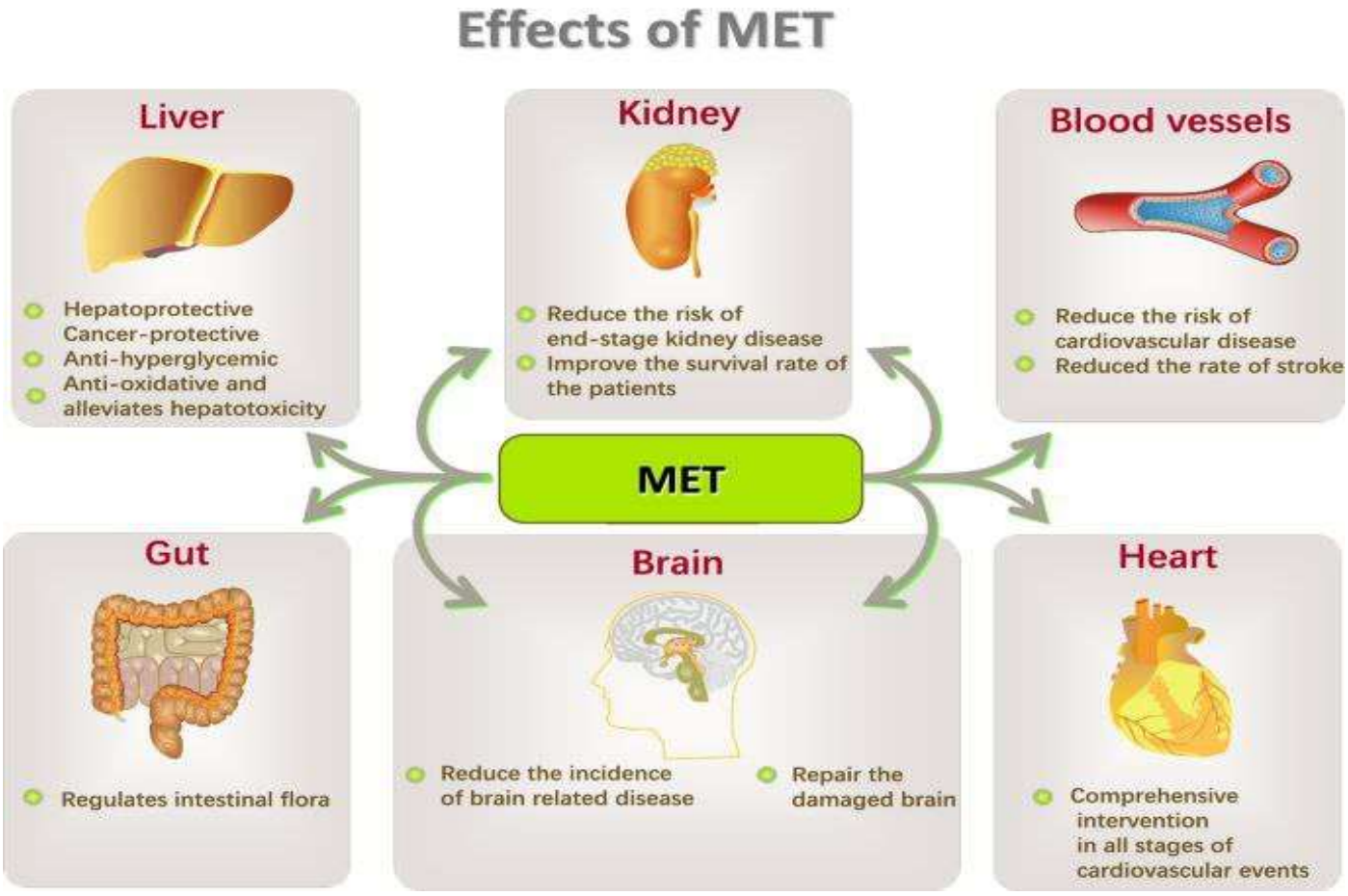


Figure -1 : Effect of Metformin on Human Body

Metformin in therapeutic applications in human diseases: its mechanism of action and clinical study[28]

Glycemic effects of metformin :

In use for over 30 years, metformin is the most commonly prescribed oral anti- hyperglycemic and is the first-line treatment for T2D. It acts primarily by decreasing hepatic glucose production, with additional effects by decreasing intestinal absorption of glucose and improving peripheral glucose uptake, Hypoglycemia is unlikely with metformin, making its side effects more favorable when compared to other, older oral antihyperglycemics such as sulfonylureas. In primary hepatocytes, ÓMetformin activates the AMP-activated protein kinase (AMPK) pathway, which results in the Inhibition of glucose production [29,30]. Additionally, recent advances Have shown that metformin's effects on gluconeogenesis may be independent of AMPK activation. metformin acts on the respiratory chain in mitochondria, Changing the intracellular ATP levels, thereby impairing the supply of ATP required for gluconeogenesis [29–31]. another recently reported potential target of metformin may be mitochondrial glycerophosphate dehydrogenase [32]. other mechanisms of Metformin on glycemia include potential improvements in homeostasis via actions on glucagon-like peptide 1 and antagonization of glucagon, further suppressing hepatic gluconeogenesis [33] The effect of metformin on the intestines involves several mechanisms. fundamentally, metformin decreases proximal intestinal glucose absorption, possibly by increasing enterocytic glucose utilization and increased lactate production [34].

Mechanism of Metformin in the Intestines to Control Diabetes :

The complete mechanisms by which glucose utilization is increased are unclear; however , animal models indicate a role in increased GLUT2 expression on the enterocyte membrane. The other pathways of metformin's action on the gut involve its effects on the incretin system. Metformin increases GLP-1 secretion by enteroendocrine cells In the intestine, thereby enhancing glucose homeostasis. Mechanisms of this GLP-1 Increase are under debate, and the currently prevailing opinion is that metformin acts by increasing GLP-1 production rather than by preventing its degradation by DPP-4 [35, 36]. other glucoregulatory effects via the intestines include modulations of the gut-brain axis and its effects on the intestinal microbiome [37] .Metformin reduces gluconeogenesis and hepatic glucose production, increasing peripheral glucose uptake and improving insulin sensitivity.

Metformin suppresses hepatic glucose production through the inhibition of mitochondrial respiratory chain complex 1 and AMP deaminase:

In 2000, two studies showed that metformin's action is through the disruption of mitochondrial complex I [38] leading to a decrease in ATP production. More recently, Foretz

et al. suggested that the change in the AMP/ATP or ADP/ATP ratio after metformin's inhibition of the mitochondrial complex is primarily responsible for metformin's effect and that this occurs through an AMPK independent pathway [39] . It was also shown that metformin increases AMP levels via the inhibition of AMP deaminase [40] . In addition, elevated AMP levels after metformin treatment lead to the inhibition of adenylate cyclase and a decrease in cAMP levels, resulting in the suppression of the cAMP-PKA pathway, and the inhibition of gluconeogenesis was proposed [41] . We also found that metformin is able to decrease cellular ATP levels and increase the ratios of AMP/ATP or ADP/ATP [42] . Since gluconeogenesis is an energy-demanding process in which synthesis of one molecule of glucose from lactate or pyruvate requires 4 molecules of ATP and 2 molecules of GTP, reduction of cellular ATP levels by metformin will lead to the suppression of hepatic. gluconeogenesis

Metformin suppresses hepatic glucose production through the inhibition of mitochondrial glycerol 3-phosphate dehydrogenase:

It has been reported that metformin suppresses hepatic glucose production by inhibiting the enzymatic activity of mitochondrial glycerol 3-phosphate dehydrogenase (mG3PDH), which blocks the transport of NADH from the cytoplasm into mitochondria [43]. This mechanism of metformin action may be important for the suppression of hepatic glucose production in patients with diabetes who have high levels of serum lactate, as the conversion of lactate to pyruvate leads to the generation of NADH. The inhibition of mG3PDH will result in an increase in NADH levels and a decrease in NAD+ levels, resulting in no NAD+ available for converting lactate to pyruvate. Subsequently, this action will halt glucose production from lactate interestingly, AMPK negatively regulates the activity of yeast glycerol 3-phosphate dehydrogenase 2 (an analog of mammalian mG3PDH) by phosphorylating S72. However, it remains to be determined whether AMPK can affect mG3PDH enzymatic activity by phosphorylation

Metformin alleviates hyperglycemia in T2D through the improvement of insulin signaling an indirect pathway of metformin action even though metformin improves hyperglycemia mainly through the suppression of gluconeogenesis in the liver, it has also been found to be able to increase insulin sensitivity. This effect would improve insulin-mediated suppression of hepatic glucose production and enhance insulin-stimulated glucose disposal in skeletal muscle [44]

Dosages:

The doctor usually starts by giving the patient the lowest effective dose of the diabetes regulator metformin and gradually increases the dose once every week to two weeks at most. The patient needs to monitor his blood sugar continuously so that the doctor can evaluate the work of the diabetes regulator Metformin. Metformin can be given to prevent type 2 diabetes at a dose of 850 mg per day orally, and it can be increased to 850 mg every 12 hours.

Adult dosage:

Immediate-release tablets or oral solution: Start with 500 mg every 12 hours, or 850 mg once a day with meals. The dose can be increased every two weeks to reach the appropriate maintenance dose, which is 1500 to 2550 mg per day, taken orally divided every 8 to 12 hours with meals. The dose should not exceed 2550 mg per day.Extended-release tablets: The initial dose is 500 to 1000 mg once a day in the evening, and the dose may be increased by 500 mg per day every week provided that the dose does not exceed 2000 mg per day.

Dosage for children 10 to 16 years of age with type 2 diabetes:

Immediate-release tablets or oral solution: Start with a dose of 500 mg every 12 hours, and the dose may be increased each week by 500 mg, provided that the maintenance dose does not exceed 2,000 mg per day in divided doses.Extended-release tablets: due to limited information on the effectiveness and safety of metformin, extended-release tablets should not be used in children under 17 years of age[45]

How to Use Metformin

The following instructions should be followed when using this medicine:

The patient should take metformin at around the same time each day to help him remember the medication. The patient must also follow the doctor's instructions and not take a dose more or less than the prescribed dose.

Extended-release tablets should be swallowed whole and not split, chewed, or crushed. The diabetes regulator metformin helps control diabetes, but does not cure it completely. Therefore, the patient must continue taking it even if he feels well, and treatment should not be stopped without speaking to the doctor.

If you miss a dose of metformin, you should take it as soon as you remember, but if it is almost time for the next dose, you should skip the missed dose and continue the usual dosing schedule. You should not take a double dose of the diabetes regulator to make up for the missed dose.[45]

Polycystic Ovary Syndrome (PCOS):

is the commonest endocrinopathy amongst young women, with approximately one in five women having ovaries with a polycystic appearance on ultrasound [46] and almost half of those with polycystic ovaries fulfilling the diagnostic criteria for PCOS (47)

Insulin resistance in PCOS:

Insulin resistance (IR) and secondary hyperinsulinemia affect approximately 65-70% of women with PCOS.[48,49] Many of these women are also obese, which further exacerbates

their IR. Insulin stimulates ovarian theca cell androgen production and secretion, and suppresses the hepatic production of sex hormone-binding globulin. The increased intraovarian androgen Then disrupt folliculogenesis[50] Hyperinsulinemia may also directly cause premature follicular atresia and antral follicle arrest[51] .The resulting anovulation also leads to unopposed estrogen production and endometrial proliferation in women with PCOS, leading to an increased risk of endometrial hyperplasia.

Insulin resistance appears to be the fundamental common pathway to disease amongst women With PCOS. Women with PCOS have normal insulin molecules and the insulin receptor on cell appears to be normal. However it appears to be a post-receptor deficit, in relation to the downstream cellular effects of what happens after insulin binds to the insulin receptor, meaning That the molecular cascade of intracellular events has a level of impairment, leading to a post-receptor 'intracellular' resistance to insulin. Since there is relative insulin resistance, women with PCOS produce higher levels of insulin than they otherwise would have. These increased circulating levels of insulin have direct effects on the ovaries, and the increased insulin levels also release other factors notably insulin-like growth factor 1 (IGF-1) from the liver which, in turn,exerts an effect on the ovary. The impact of higher levels of insulin and IGF-1 on the ovary is for the ovary to release higher levels of testosterone. All of these hormones including insulin, IGF-1 And testosterone prevent the growth of ovarian follicles through to ovulation, leading to an accumulation of small ovarian follicles less than 10 mm diameter that do not progress through to ovulation.

Other metabolic abnormalities commonly linked to insulin resistance are evident in patients with PCOS; these include dyslipidaemia,[50] increased concentrations of tissue plasminogen activator,[51] and low-grade chronic inflammation.[52] In line with these metabolic features is emerging evidence that patients with a history of PCOS,

PCOS is also a leading cause of female hyperandrogenism and a state of relative estrogen excess[53,54]. The disorder often coexists with prolactin excess, although there are no convincing data that they are causally linked with each other [55] . Because of frequent disturbances in glucose homeostasis, women with PCOS often need to be treated with metformin

Long-term prolactin excess is often complicated by obesity/overweight, insulin resistance and impaired glucose tolerance [56] disturbances commonly treated with metformin [57]. In numerous Studies, metformin inhibited enhanced secretory function of human lactotropes [58–59] . The drug reduced prolactin levels, irrespective of the reason for prolactin excess

The degree of reduction in prolactin levels depended on metformin dose and was most pronounced in individuals receiving high-dose treatment (2.55–3 g daily) [60]. The impact of Metformin on prolactin concentration was also determined by sex and was statistically significan only in women [61]. Metabolic disturbances resulting from prolactin excess are

effectively reversed by dopaminergic agents; however, owing to intolerance or contraindications, not all patients can be treated with these agents [62]. Some of these patients, particularly women with coexisting disturbances of glucose homeostasis, may be candidates for metformin treatment

The decrease in women with PCOS, accompanied by a decrease in the LH/FSH ratio, which is in line with previous observations of other research teams [63,64], is probably a consequence of the inhibitory effect on overactive gonadotropes. It was found that the inhibitory effect of metformin on gonadotropin secretion was mediated by pituitary AMPK, and gonadotropes were pituitary cells with abundant expression of this enzyme [65]. The presence of correlations between the impact metformin on LH and the LH/FSH ratio and on testosterone and FAI indicates that the decrease in LH secretion in this group of women contributed to a reduction in testosterone production. In turn, The increase of LH in the control group is probably secondary to the decrease in prolactin levels. This explanation is supported by the presence of correlations between the impact on prolactin and on LH in the control subjects but not in women with PCOS. Through inhibition of hypothalamic Gonadotropin-releasing hormone pulsatile secretion, prolactin excess reduces gonadotropin Secretion and may have an additional direct inhibitory effect on ovarian function [66]. Because the decrease in prolactin and the subsequent increase in LH in subjects without PCOS were only moderate, an indirect effect on ovarian function may be counterbalanced by a direct inhibitory effect of this agent on ovarian steroidogenesis

Unfortunately, there are no head-to-head studies comparing the prolactin-lowering properties of monotherapy with a dopamine agonist and metformin. However, unlike metformin recipients in the current study, bromocriptine [67.68] and cabergoline [69] monotherapy lead to a significant decrease in prolactinin hyperprolactinemic women with concomitant PCOS. Furthermore, cabergoline administered to women with this syndrome together with low-dose metformin was superior to low-dose metformin alone in reducing prolactin levels [70,71]. These findings indirectly indicate that also in women with PCOS, dopaminergic agents are more potent in reducing monomeric hyperprolactinemia than metformin.

Treatment with Metformin:

Metformin is currently the most widely used drug worldwide for the treatment for type 2 DM. Its primary action appears to be an inhibition of hepatic glucose production and an increase in peripheral insulin sensitivity. The benefits of metformin on insulin sensitivity have been demonstrated in non-DM women with PCOS. The use of metformin is associated with increased menstrual cyclicity, improved ovulation, and a reduction in circulating androgen levels.[72] Metabolic benefits are enhanced in the presence of weight loss, and weight loss itself may be enhanced in the presence of metformin.[73] below, we discuss further the mechanisms of action of metformin and its clinically relevant role in the treatment of PCOS.

Metformin Alone for the treatment of subfertility:

Metformin likely plays its role in improving ovulation induction in women with PCOS through a variety of actions, including reducing insulin levels and altering the effect of insulin on ovarian androgen biosynthesis, theca cell proliferation, and endometrial growth. Also, potentially through a direct effect, it inhibits ovarian gluconeogenesis and thus reduces ovarian androgen production.[74,75] In determining which clinical parameters may predict which patients will benefit most from metformin for ovulation induction, fasting insulin levells and glucose to insulin ratios do not predict the ovulatory response to metformin.[76]

Metformin in combination with clomefin citrate for the treatment of subfertility:

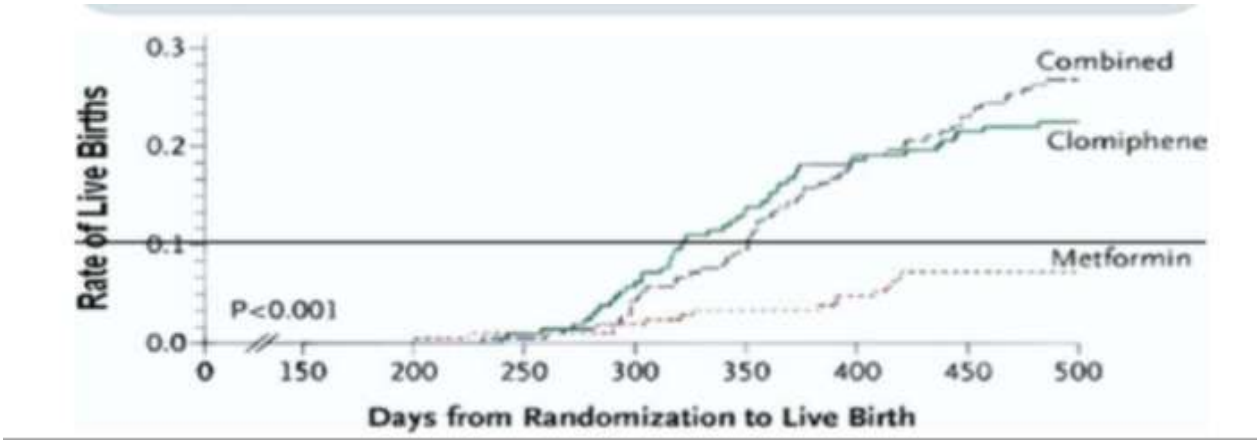
Metformin has been suggested for the treatment of PCOS oligoovulatory infertility, either alone , or in combination with dietary restriction , Clomephine citrate, or gonadotropins. In the PCOS trial [67] reported that metformin alone was significantly less successful than the combination of clomephine citrate and metformin (live birth rates 7.2% vs 26.8%)

However, the combination of metformin and clomephine citrate (CC) was not significantly different from the rate of CC alone؛ It is possible that women who have failed to ovulate with CC (ie, CC-resistant) may benefit from the addition of metformin. Even though the reason for the ovulatory resistance to CC has not been clearly identified, it can be hypothesized that metformin therapy would augment the induction of ovulation in CC-resistant women because of its favorable change in androgens, gonadotropins, and insulin, through mechanisms distinct from those of CC.[63] It is plausible to assume that women with CC resistance receiving metformin have an increased response to CC secondary to an intrinsic alteration of the micro-environment of the follicle caused by the effect of metformin pretreatment on insulin and the insulin growth factor (IGF)-I pathway in granulosa cells.[64,66]

Metformin in Infertility :

Metformin as pretreatment and cotreatment with clomiphene citrate seems successful, perhaps by sensitising follicles to follicle-stimulating hormone (FSH). Therefore, metformin alone and later in combination with clomiphene citrate has been proposed as a sequential treatment programme before the use of gonadotropin therapy for ovulation induction in infertile women with PCOS [63,64]

Metformin also improved responses to ovulation induction with exogenous FSH stimulation.women with clomiphene-resistant PCOS received daily metformin (1000–1500 mg) in half of 60 cycles before gonadotropin treatment. The total number of follicles on the day of treatment with human chorionic gonadotropin was lower in the women given metformin than in those who did not receive it, but there were more mature oocytes and both



the fertilisation rate and the clinical pregnancy rate were significantly higher.[77]

Kaplan-Meier curves for live birth. According to Study group in Pregnancy in Polycystic Ovary Syndrome (PCOS) trial, in which 676 infertile Women with PCOS, ages 18-39 years, with no other infertility factors, were randomized to 3 Treatment arms for total of 6 cycles or 30 weeks: Metformin (1000 mg twice a day) placebo(PBO); clomiphene citrate (CC) (50 mg/d) for 5 Days (day 3-7 of cycle) PBO; and combination of metformin CC. Adapted from Legro et al [67]

Antimicrobial Activity of Metformin:

Metformin can exert Adjuvant antimicrobial activity through several possible mechanisms, including potentiation of the activity of the antibiotics, modifying the immune response of the host cells to the infection, and its ability to increase the intra cellular accumulation of different antibiotics through disrupting the outer membranes of bacteria

The gut microbiota is a diverse and dynamic community of micro-organisms that inhabit the gastrointestinal tract and have a significant impact on human health and disease [78] These microorganisms play a vital role in the digestion and absorption of nutrients, the regulation of the immune system, and the production of essential metabolites. The composition and function of the gut microbiota are influenced by a variety of factors, including diet, lifestyle, and medication use [79]. Metformin is a first-line medication widely used for the treatment of type 2 diabetes [80]. In recent years, there has been growing interest in the potential effects of metformin on the gut microbiota [81,82] . Preclinical Studies have demonstrated that metformin alters the gut microbiota composition and function, and there is evidence to suggest that these changes may be beneficial for metabolic and immune health [83, 84] Despite the growing interest in this topic, the impact of metformin on the gut microbiota in humans

remains unclear [85]. While some Studies have reported significant changes in the gut microbiota following metformin treatment, others have found no significant effects [86_87]

Metformin's action mechanism is associated with physiological processes in the gastrointestinal tract. For example, a more pronounced effect of metformin can be observed when the drug is administered orally than intravenously at an equivalent dose (83). It has been estimated that 20-30% of people receiving Metformin therapy develop gastrointestinal side effects, with approximately 5% being unable to tolerate metformin at all [88].Metformin accumulates in gastrointestinal tissues [89] ; for Example, it is 30-300 times more concentrated in the small Intestine than in plasma, and 30-50% of the drug reaches the colon and is eliminated with feces [84] . Studies in humans have convincingly shown that metformin Specifically alters gut microbiome both in T2D patients [85,90] and healthy subject

Antibacterial activity of Metformin:

Adjuvants effect of against Gram-positive and Gram-negative bacteria. In Addition, it is applied to various biological mechanisms, including alteration of the immune Response to infection, potentiation of antibiotic activity, and the ability to act by destroying The outer membranes and efflux pumps of bacteria. For example,

- metformin has the potential to increase the efficacy of anti-TB by enhancing Autophagy [91]

- In another study metformin restores antibiotic susceptibility Through intracellular accumulation of doxycycline in tetracycline-resistant *Escherichia coli* [92]

-In addition, metformin was found to target the outer membrane of Gram-negative Bacteria, thus inhibiting the growth of *Klebsiella pneumoniae*

Metformin can inhibit mitochondrial respiratory-chain complex-1 and activate the Adenosine monophosphate (AMP)-activated protein kinase (AMPK) pathway to Facilitate neutrophil activation, chemotaxis, and bacterial killing [93,94].

Metformin can improve T and B-cell function in patients with obesity and T2DM [94,95]. Metformin can decrease pro-inflammatory markers of C-reactive protein, interferon-α [96], tumor necrosis factor-α, and interleukin-6, and increase the level of the anti Inflammatory marker IL-10 [93,94].

The inhibition of mitochondrial complex-1 and electron transport by metformin can decrease the energy supply required for bacterial growth. Metformin can also inhibit bacterial gluconeogenesis, and limited glycerol use in the krebs cycle can decrease bacterial virulence. The anti-folate effect of metformin may inhibit the bacterial folate cycle and suppress bacterial growth [97].

Metformin decreases the expression of nitric oxide synthase and ameliorates vasodilatation with anti-endotoxaemic and vasoactive properties [98]

Patients with T2D are at high risk of pneumonia and other respiratory infections including chronic obstructive pulmonary diseases (COPD). In patients with community- acquired pneumonia, increased neutrophil-to-lymphocyte ratios indicating a heightened pro-inflammatory state had been associated with poor outcomes. In a cohort of 3537 patients with T2D, long-term treatment with metformin was associated with reduced neutrophil-to-lymphocyte ratios,

The Role of Metformin in Preventing the Development and Progression of Bacterial *Pneumonia*:

Metformin inhibits mitochondrial respiratory-chain complex-1 and activates the liver kinase B1 (LKB1)/ AMPK pathway to facilitate neutrophil-dependent bacterial uptake and killing, and promote innate immune response[18] , but it can suppress pro-inflammatory markers of high sensitiv- ity C-reactive protein,interferon-α(IFN-α)[99], tumor necrosis factor-α (TNF-α), and interleukin -6 (IL-6); and inhibits neutrophil activation and chemotaxis, improves neutrophil to lymphocyte ratio[100], reduces B-cell intrinsic inflammation, increases antibody response, and stabilizes mast cells18. Metformin also can boost levels of the anti-inflammatory marker IL-10[100]

The inhibition of mitochondrial complex-1 and electron transport can also suppress the energy production required for bacterial growth. Metformin inhibits bacterial gluconeogenesis, and the limited utilization of glycerol in the kreb's cycle reduces bacterial virulence; the anti-folate effect of metformin may inhibit the folate cycle of bacteria and limit bacterial growth[101] . Thus, metformin may attenuate the risk of *bacteria pneumonia* by its metabolic, immunologic, and antibacterial effects.

Klebsiella pneumoniae (K. pneumoniae), an encapsulated, Gram-negative non- motile bacterial pathogen. It is a member of the normal gastrointestinal tract and nasal flora and causes no diseases in healthy individuals, but it is an opportunistic pathogen that causes several types of infections in immu- nocompromised individuals [102. 103]. *K. pneumoniae* may cause meningitis, bloodstream infections, and surgical site infections [104] Five virulence factors (biofilm formation, urease, proteases, hemolysins production, and resistance to oxidative stress) were assessed in *K. pneumoniae* in the presence and absence of 1/8 MIC (1 mg/ml) for the tested potential inhibitors Interestingly, the tested potential inhibitors had a significant inhibitory effect on the production of virulence factors.

Metformin is proven to be used as a newer efflux pump inhibitor in *K. pneumoniae* , and as a quorum sensing (QS) inhibitor in P. aeruginosa [105]

Metformin is safe and effective in treating some health problems in neonates which was tested as anti-virulence agents against neonates isolates of MDR *K.pneumoniae.* Metformin has been shown to maintain maternal glycemic control in a previous study. Fetal and placental tissues

contain clinically relevant levels of metformin (50%-100%) [106] . According to meta analysis study [107], metformin may have the same glycemic control as insulin and may be the most effective drug for preventing maternal and neonatal complications.

By the current results, previous studies revealed that metformin significantly reduced the production of the virulence factors proteases and hemolysins in another Gram-negative bacteria *Pseudomonas aeruginosa* which shows a promising anti-virulence activity of metformin [108].

The Role of Metformin on *Escherichia coli*:

E.coli is a bacterium that is commonly found In the gastrointestinal tract of humans and warm-blooded animals.due to its high prevalence in the gut, *E. coli* is used as the preferred indicator to detect and measure faecal contamination in the assessment of food and water safety . Pathogenic *E. coli* are distinguished from other *E. coli* by their ability to cause serious illness as result of their genetic elements for toxin production, adhesion to and invasion of host cells, Interference with cell metabolism and tissue destruction

Recently metformin has been suggested to act on transient receptor potential ankyrin 1 (TRPA1) channels [109] . TRPA1 is known to be present in bladder epithelial cells[110] and is activated upon binding to the *E. coli*, That metformin acts as a TRPA1 inhibitor in uroepithelial cells, consistent with a previous report in neurons[109] . Metformin upregulated TRPA1 mRNA and protein expression in telomerase immortalized normal human urothelial cells (TERT-NHUC) , with prominent enrichment of TRPA1 specifically in the lysosomes but not in other organelles such as mitochondria

Metformin stimulated expression of the antimicrobial peptides LL-37 and Rnase, respectively. The result of our data show that metformin treatment stimulates multiple host-protective responses, resulting in increased intra- and extracellular *E. coli* killing.

The Role of Metformin on *Pseudomonas aeruginosa*:

metformin markedly decreased biofilm formation, and increased the sensitivity to oxidative stress [111] . Also, metformin inhibits biofilm formation, proteases production, and hemolysins production in Serratia marcescens[112]

The inhibition of biofilm formation was evaluated using the crystal violet method. Interestingly, metformin significantly reduced the biofilm formation ability of *K. pneumoniae*. The percentages of biofilm formation were reduced by a percentage ranging between (22%-68%) in metformin

Quorum sensing controls of the production of *P. aeruginosa* virulence factors such as elastase, protease, hemolysin, pyocyanin, in addition to swimming and twitching motilities, biofilm formation and resistance to oxidative stress, Metformin significantly reduced the production of

violacein pigment. Significant inhibition of pyocyanin, hemolysin, protease and elastase was achieved .

Metformin markedly decreased biofilm formation, swimming and twitching motilities and increased the sensitivity to oxidative stress. In the molecular docking study, metformin could bind to type of quarun sensing by hydrogen bond [113]

Materials and Methods:

2. Materials and Methods:

2.1. Collection of Bacterial Samples : eighty seven samples collected(for period from October 2023 to February -2024) from patients (female) suffering from vaginal infections who attending the gynecology clinic in Gynecology and obstetrics teaching hospital in Hillah, those patients ranged (19-50) years old, multiple clinical data from patents were collected according to data sheet(questioner) such as age, metformin present in therapy, Diabetic Mellitus and family history of chronic diseases, active bacterial infections in addition to some other information, then diagnosed causative bacteria (aerobically only), the identification and diagnosis tests performed for all bacterial isolates including biochemical tests, culture of isolates were used, then all isolated bacteria tested for antibiotic sensitivity test, MacConkey agar used for isolation *Enterobacteriaceae* , nutrient agar media for isolation of *P. aeroginosa* and Mulller-Hinton agar for Antibiotics sensitivity tests.

2.2 Metformin as Adjuvant to Antibiotics:

Dilute metformin 500 mg with 10 ml distilled water (D.W.) then diffuse diluted metformin by using sterile swab on Mulller-Hinton agar, let it dry completely, then perform Kirby bour method by using the same tested antibiotic discs after culturing isolated bacteria of (*E.coli, K. pneumoniae* and *P. aeroginosa*), then let the cultured media in 37˚C for 18-24hr, after that read the results by measuring of inhibition zone diameter.

Results:

The present study indicated for some findings appeared as in tables and figures below:

Table-1 Demographic Characteristics of Patients (Part-1)

Patient (87)		
Age	(19-30)	46 **(52.8%)**
	(30-40)	35**(40.2%)**
	(40-50)	6 **(6.89%)**
Married / unmarried		82 / 5 **(32.1% / 5.7%)**
Age of marriage		19-37 **(21.8% /42.5%)**
Type of delivery : Cs / Normal		78 /9 **(89.6% / 10.3%)**

Table-2 Demographic Characteristics of Patients (Part-2)

Patient (87)		
Weight		45-92 **kg**
Level of education	Primary	6 **(6.89%)**
	Secondary	19 **(21.8%)**
	Postsecondary	62 **(71.2%)**
Occupation	House Wife	20 **(22.98%)**
	Student	26 **(29.8%)**
	employee	41 **(47.1 %)**
Residence: Urban / Rural	87/0 **(100%)**	

Table-3 Demographic Characteristics of Patients (Part-3)

Patient (87)		
Family History of Disease	70/ No (80.4 %)	* 4 / Diabetes & Hypertension **(4.9 %)** * 11/Diabetes **(12.6 %)** * 2 / Thyroid **(2.2 %)** **Total: 17 (19.5 %)**
Polycystic Ovary Syndrome (POS)	46/ yes **(52.8%)**	41/ No **(47.1 %)**
Hypertension	85/ No **(97.7%)**	2/ Yes**(2.2 %)**
Heart Disease	86/ No **(98.8 %)**	1/ Arrhythmia**(1.1 %)**
Cigarette Smoking	87/ No **(100 %)**	0/No **(0 %)**

Table-4 Diabetes Characteristics of Patients (Part-4)

Patient (87)			
Diabetes	24/ Yes **(27.5 %)** * 20 with POS **(83.3 %)**	63 / No **(72.4 %)**	
Diabetes during pregnancy	11 / Yes **(12.6 %)**	76/ no **(87.3 %)**	
Date of having diabetes	4 **(4.5 %)** 3- 5 month	15 **(17.2%)** 1-5 years	5 **(5.7 %)** 5-8 years

Table-5 Infection and Infection Types

Patient (87)		
Infection	84/ Yes **(96.5 %)**	3 / No **(3.4%)**
Type of infection	53 / Bacterial **(60.9 %)**	31/ No Growth **(35.6 %)**

Table-6 Bacterial Etiology of vaginal infecti

Patient (87)			
Bacterial Etiology of infection	***E.coli***	***K. pneumoniae***	***P. aeroginosa***
Isolates Count	25 **(47.1%)**	19 **(35.8 %)**	9 **(16.9 %)**
Total Bacterial Infections	**53**		

Table-7 Metformin in Therapy and Period

Patient (87)		
Metformin present in therapy	48/ Yes**(55.1 %)**	39/ No **(44.8%)**
First time to use metformin		
1-6 months	11**(12.6 %)**	
6-12 months	32**(36.7 %)**	
12- 24 months	5**(5.7 %)**	
Total	48**(55.1 %)**	

Table-8 Antibiotic Resistance of bacterial isolates (*k.pneumoniae*)- (Antibiotic only, Antibiotic with Metformin)

Bacteria	Antibiotic Type	Inhibition zone of Antibiotics only (mm)	Inhibition zone of Antibiotics with metformin (mm)
k.pneumoniae	**CIP**	20	27
	CTX	16	20
	AK	17	20
	GEN	19	28
	F	15	16
	AMC	0	23
	CAZ	0	0
	FOX	0	0
Only six antibiotics indicated increased inhibition zone in presence of metformin **75 %**			

* **CIP** = Ciprofloxacin

CTX = Cefotaxime

AK = Amikacin

GEN = Gentamicin

F = Flucloxacillin

AMC = Amoxicillin-clavulanic acid

CAZ = Ceftazidime

FOX = Cefoxitin

Table-9 Antibiotic Resistance of bacterial isolates (*P.aeroginosa*)-(Antibiotic only, Antibiotic with Metformin)

Bacteria	Antibiotic Type	Inhibition zone of Antibiotics only(mm)	Inhibition zone of Antibiotics with metformin (mm)
P.aeroginosa	**CIP**	25	30
	CTX	0	10
	AK	30	34
	GEN	30	38
	F	30	34
	AMC	0	20
	CAZ	0	0
	FOX	0	0
Only six antibiotics indicated increased inhibition zone in presence of metformin **75 %**			

*** CIP** = Ciprofloxacin

CTX = Cefotaxime

AK = Amikacin

GEN = Gentamicin

F = Flucloxacillin

AMC = Amoxicillin-clavulanic acid

CAZ = Ceftazidime

FOX = Cefoxitin

Table-10 Antibiotic Resistance of bacterial isolates (*E.coli*)- (Antibiotic only, Antibiotic with Metformin)

Bacteria	Antibiotic Type	Inhibition zone of Antibiotics only(mm)	Inhibition zone of Antibiotics with metformin (mm)
E.coli	**CIP**	20	20
	CTX	26	22
	AK	28	25
	GEN	28	20
	F	23	25
	AMC	12	10
	CAZ	0	0
	FOX	0	0
Only one antibiotic indicated increased inhibition zone in presence of metformin **12.5 %**			

* **CIP** = Ciprofloxacin

CTX = Cefotaxime

AK = Amikacin

GEN = Gentamicin

F = Flucloxacillin

AMC = Amoxicillin-clavulanic acid

CAZ = Ceftazidime

FOX = Cefoxitin

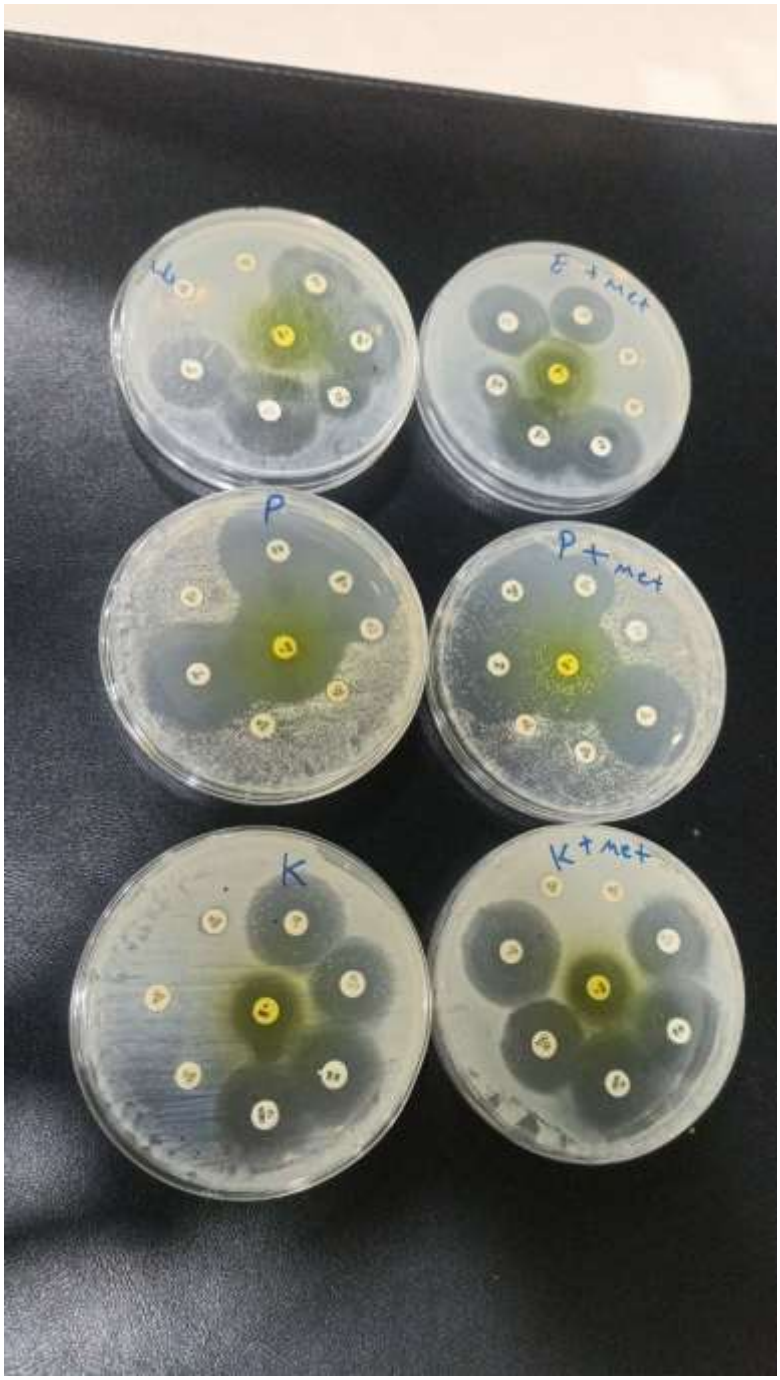


Figure-(2)Antibiotic Susceptibility tests (Antibiotic only, Antibiotic with Metformin)on Isolated Bacteria

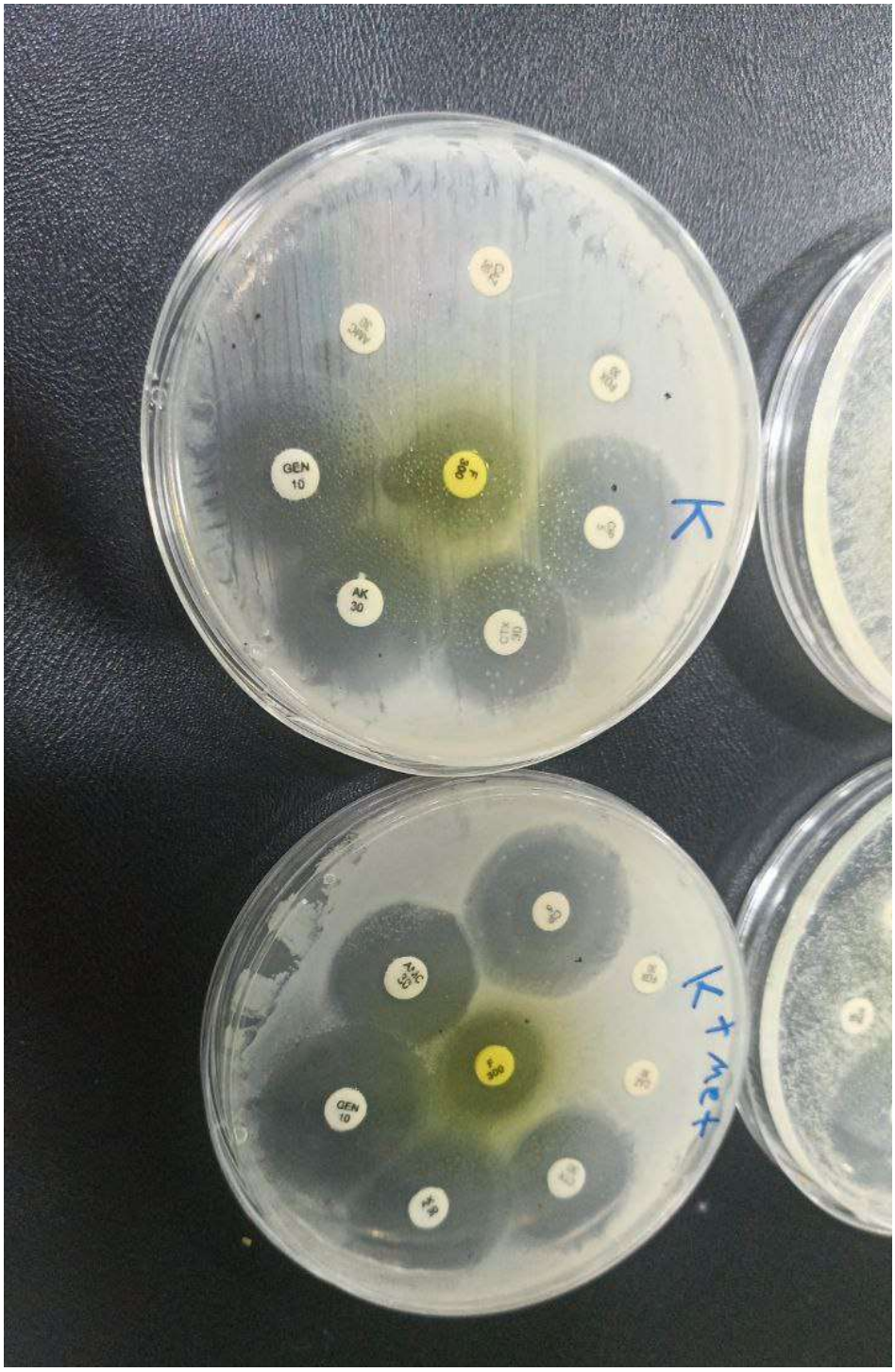


Figure-(3)Antibiotic Susceptibility tests (Antibiotic only, Antibiotic with Metformin)on *Klebsiella pneumoniae*

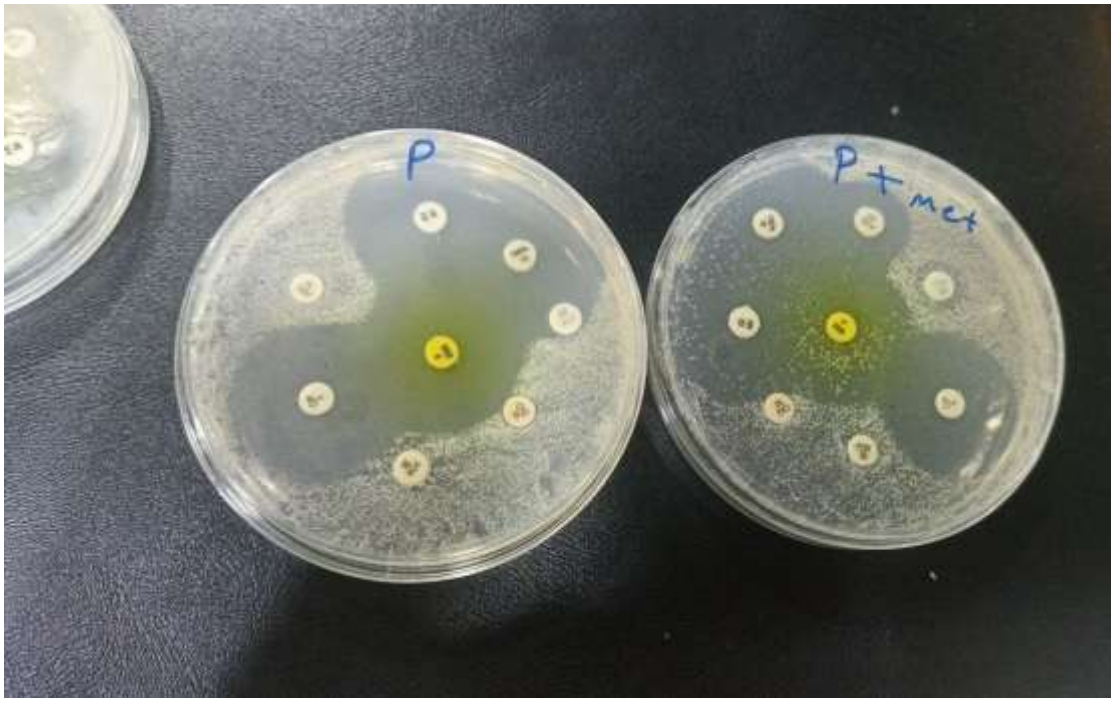


Figure-(4)Antibiotic Susceptibility tests (Antibiotic only, Antibiotic with Metformin)on *Pseudomonas aeroginosa*

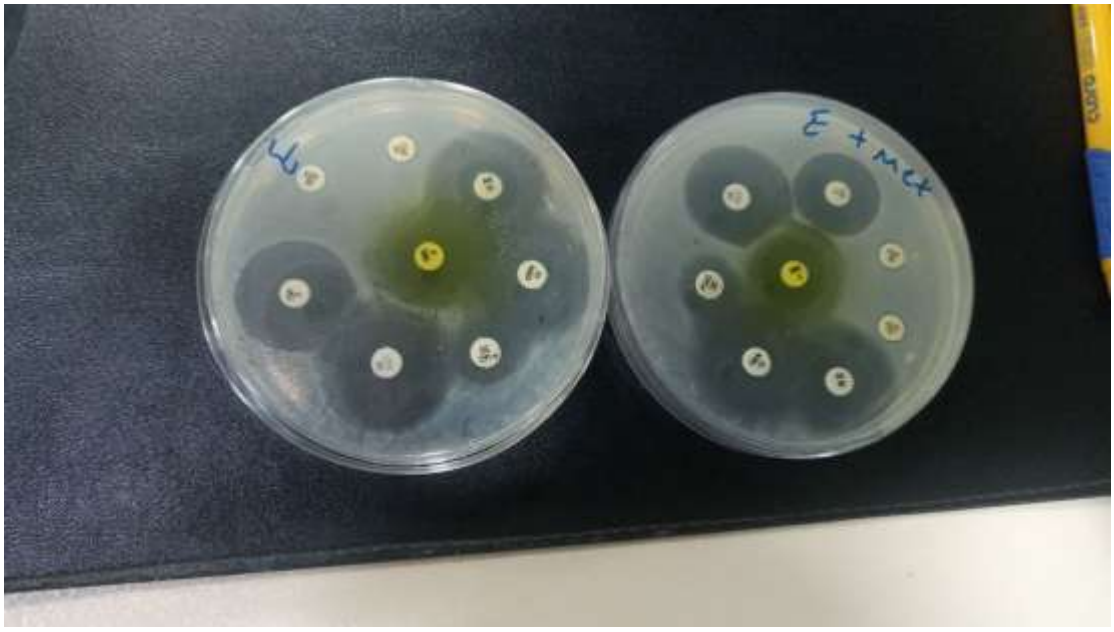


Figure-(5) Antibiotic Susceptibility tests (Antibiotic only, Antibiotic with Metformin)on *E.coli*

Discussion:

In recent years, the emergence and fast spread of antibiotic resistance has become a severe threat to health all over the globe. Classical methods that were aimed to contain or slow the rapid progression of bacterial resistance through better antibiotic prescribing policies, have become insufficient at the global level. Urgent measures along with identification of novel adjuvant strategies to restore the efficacy of the preexisting antibiotics and ensure better clinical outcomes, in a cost-effective manner, are highly needed [114].

Results of current study indicated isolation of *E.coli, K. pneumoniae* and *P.aeroginosa* only as a etiology of vaginal infections from female aged (19-50)years, some cultured indicated negative results (no growth) as appeared in (Table-5), this may interpreted as the causative agent may be other microorganisms, fungal ,viral or parasites, or it may attributed to anaerobic bacteria. Moreover, the present research revealed that metformin effect on *K.pneumoniae* and *P. aeroginosa* was clearly increased compared to effect of antibiotics only, while regarding *E.coli*, the effect of metformin mixed with tested antibiotics was moderate and lower obvious than on other isolated bacteria.

In certain study (which is roughly in consistent with results of the present study) revealed isolation of bacteria, *Candida* spp, *Trichomonas* spp., the major pathogens were *E.coli* (15%), *K.pneumoniae* (2%), in addition to isolation of *S.aureus* (9%) and *Candida* (16%). *Lactobacilli*, *B.fragilis* and *Peptostreptococcus* spp. were the anaerobes isolated. *E.coli*, *S.aureus*, *Candida* spp. were 18%, 12%, 18% reported in diabetic women and 12%, 6%, 14% reported in non-diabetic women respectively [115] Consistent with our findings, several previous published studies showed that metformin has the potential to be used in combination with other antibiotic [116,117, 118] and metformin can reduce the resistance of bacteria and efficiently restore the efficacy of the antibiotics [119]. These studies along with other studies confirm the antimicrobial potential of metformin either alone or in combination with antibacterial drugs [120,121,122].

Conclusion:

In summary, metformin, in combination with other antibiotics, exerts an antimicrobial effect when studied on different bacterial strains. It may be promising to consider metformin as a new assistant therapy to tackle bacteria.

Recommendations:

Several studies are still needed for better understanding the mechanisms underlying this preventive effect of metformin, but the preliminary findings from our study highly support the use of metformin as an adjuvant to antibiotics to reduce bacterial resistance especially on *Klebsiella pneumoniae* and *Pseudomonas aeroginosa* and lower effect on *E.coli* .

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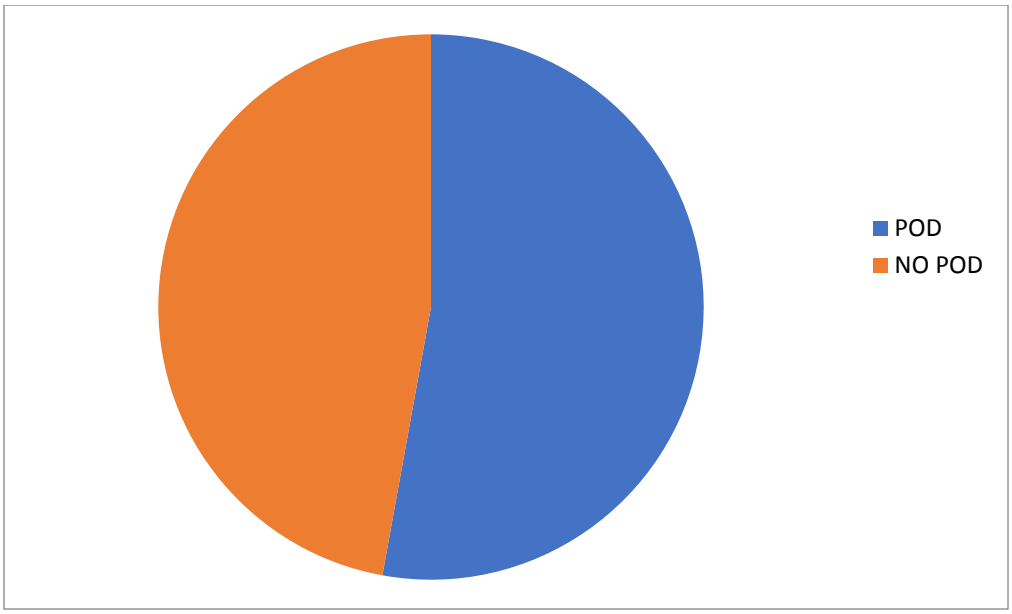

Figure-(6) Pie Chart for Presence of Pod (PCOS)

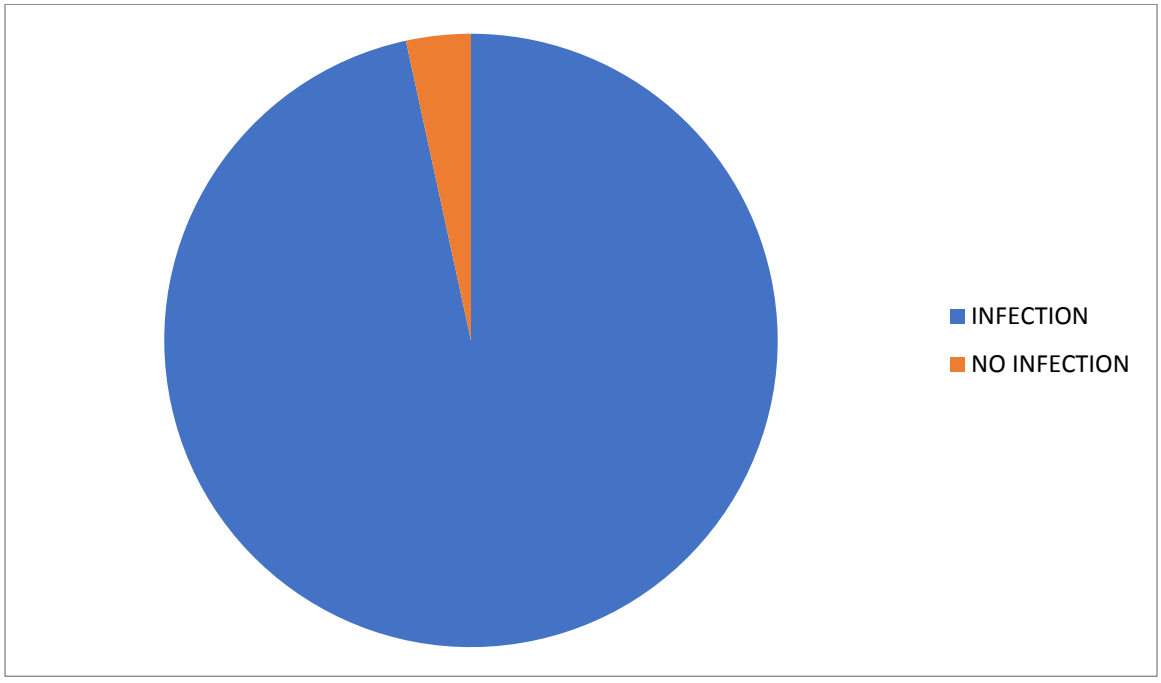

Figure-(7) Pie Chart for Presence of Infections

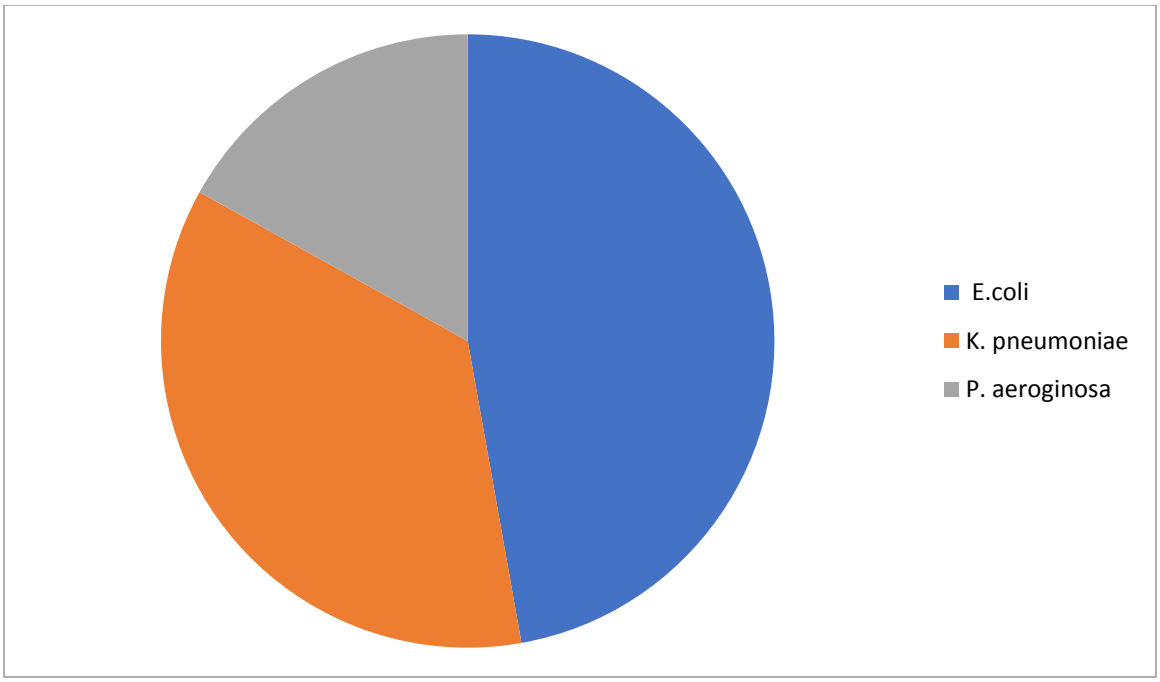

Figure-(8) Pie Chart for Type of Bacterial Infections

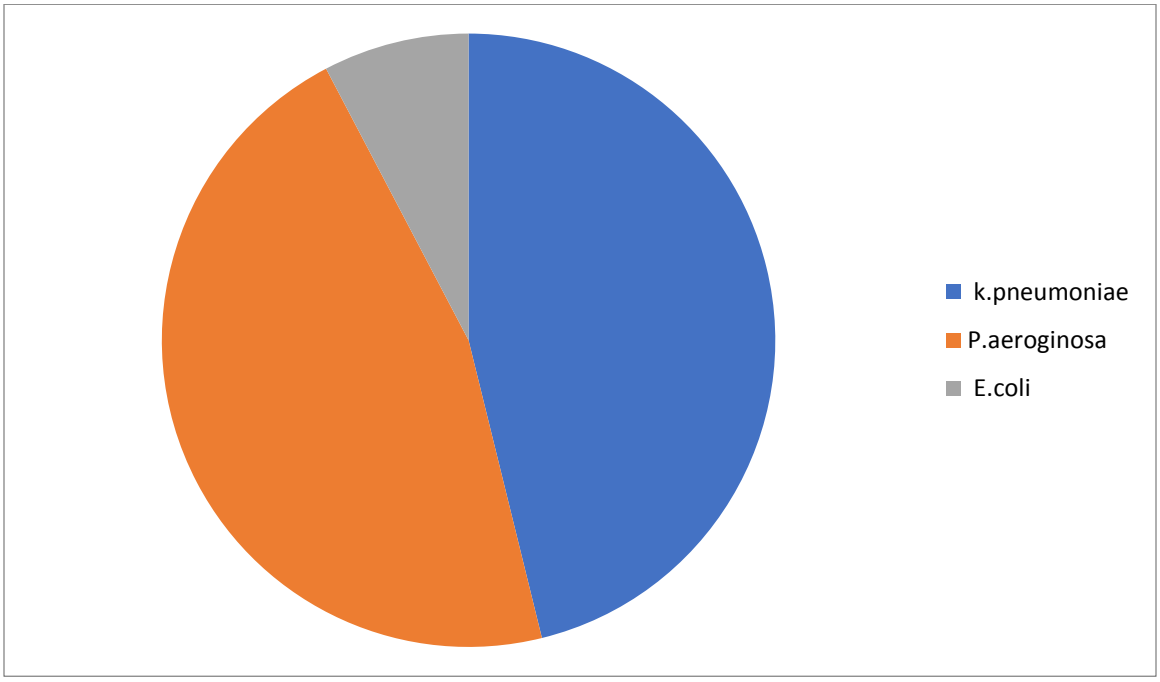

Figure-(9) Pie Chart for Adjuvant Antibacterial Effect of Metformin with Each Type of Isolated Bacteria